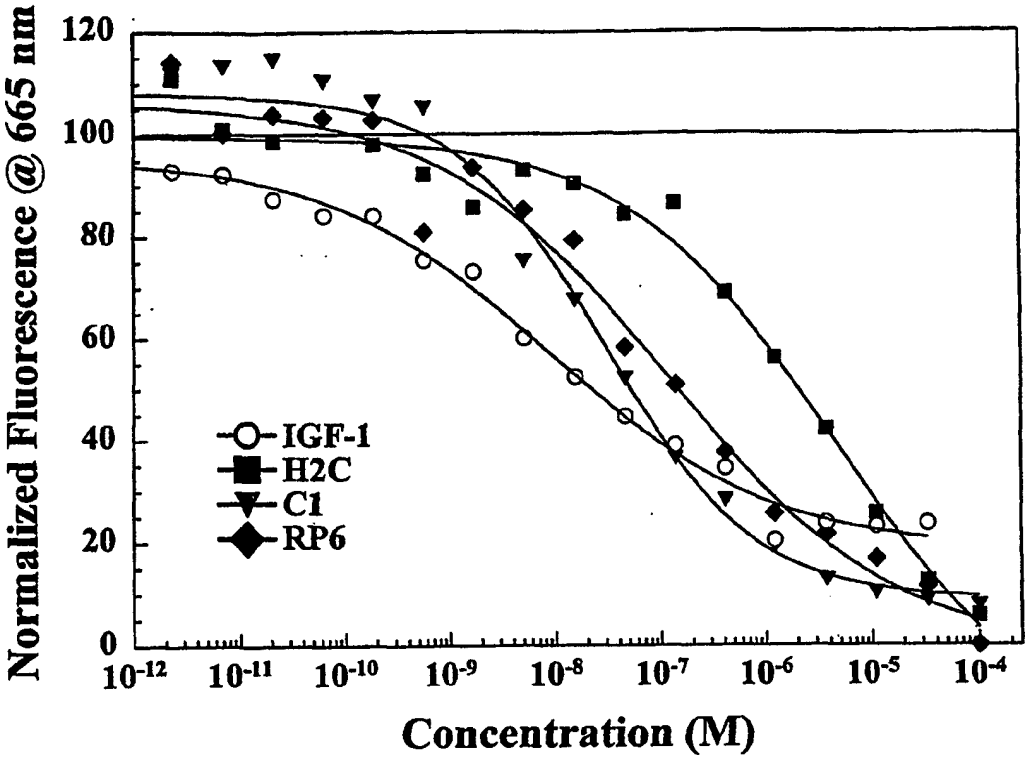

Normalized Fluorescence @ 665 nm
120
100
80
60
40
20
0
IGF-1
H2C
C1
RP6
10-12
10-11
10-10
10-9
10-8
10-7
10-6
10-5
10-4
Concentration (M)

FIG. 73

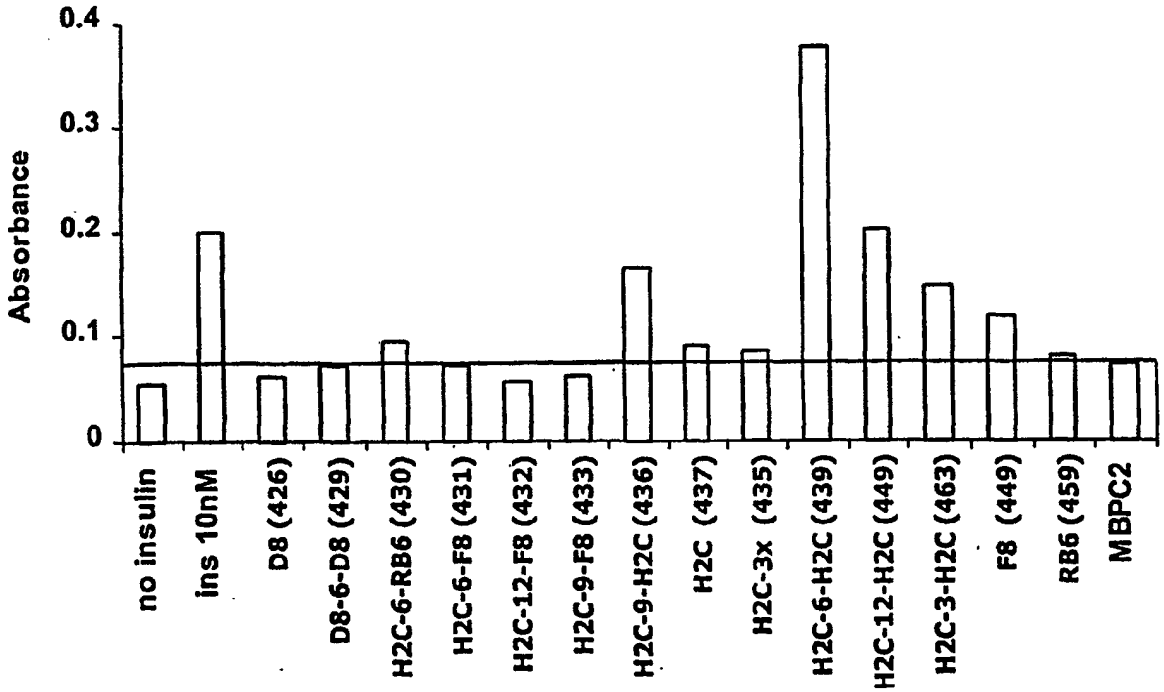

Absorbance
0.4
0.3
0.2
0.1
0
no insulin
ins 10nM
D8 (426)
D8-6-D8 (429)
H2C-6-RB6 (430)
H2C-6-F8 (431)
H2C-12-F8 (432)
H2C-9-F8 (433)
H2C-9-H2C (436)
H2C (437)
H2C-3x (435)
H2C-6-H2C (439)
H2C-12-H2C (449)
H2C-3-H2C (463)
F8 (449)
RB6 (459)
MBPC2

FIG. 74

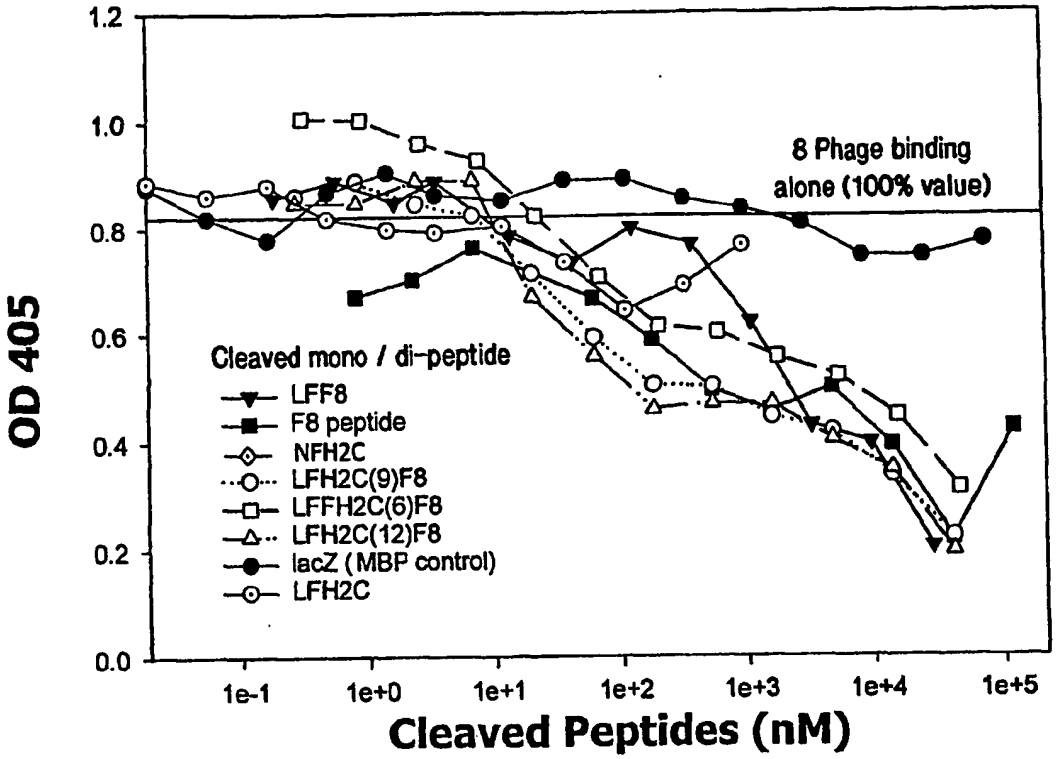


FIG. 72A

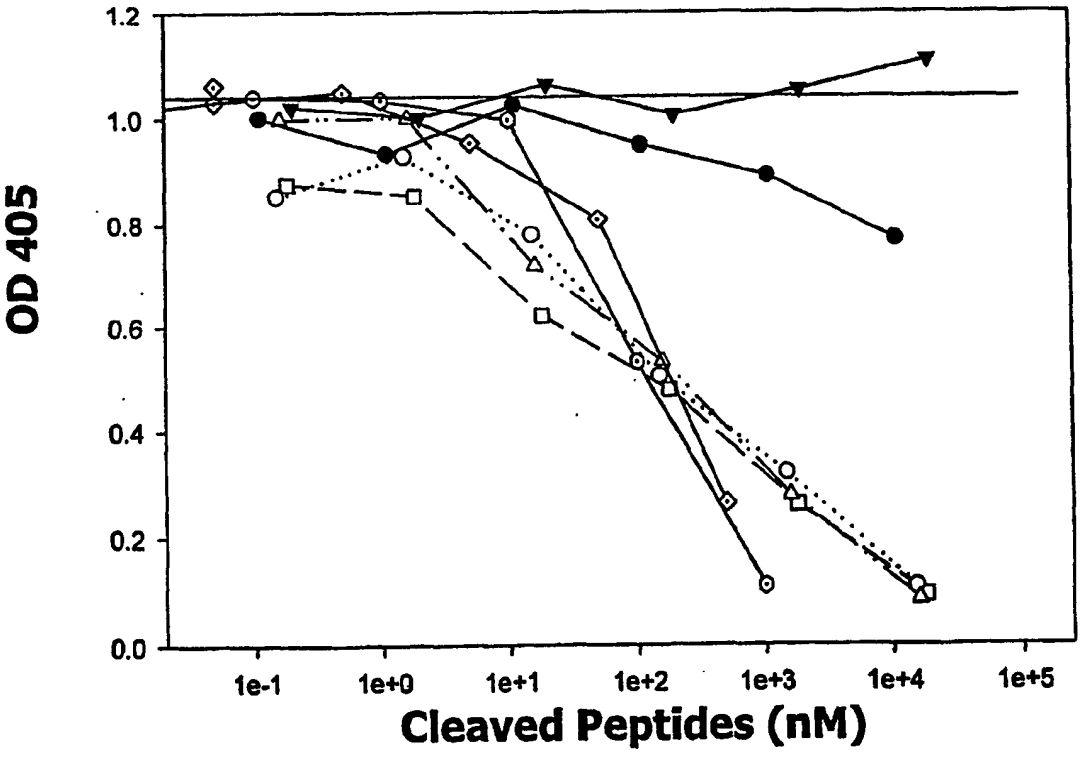
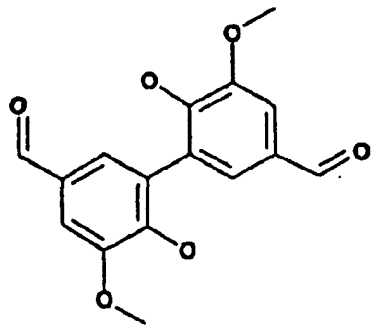


FIG. 72B

S291: Dimer of S204 with linker 9

S204 = Lig-GGGFHENFYDWFVRQVSKK

Linker 9 = 

HIR binding = $1.2*10^{-6}$

FFC:

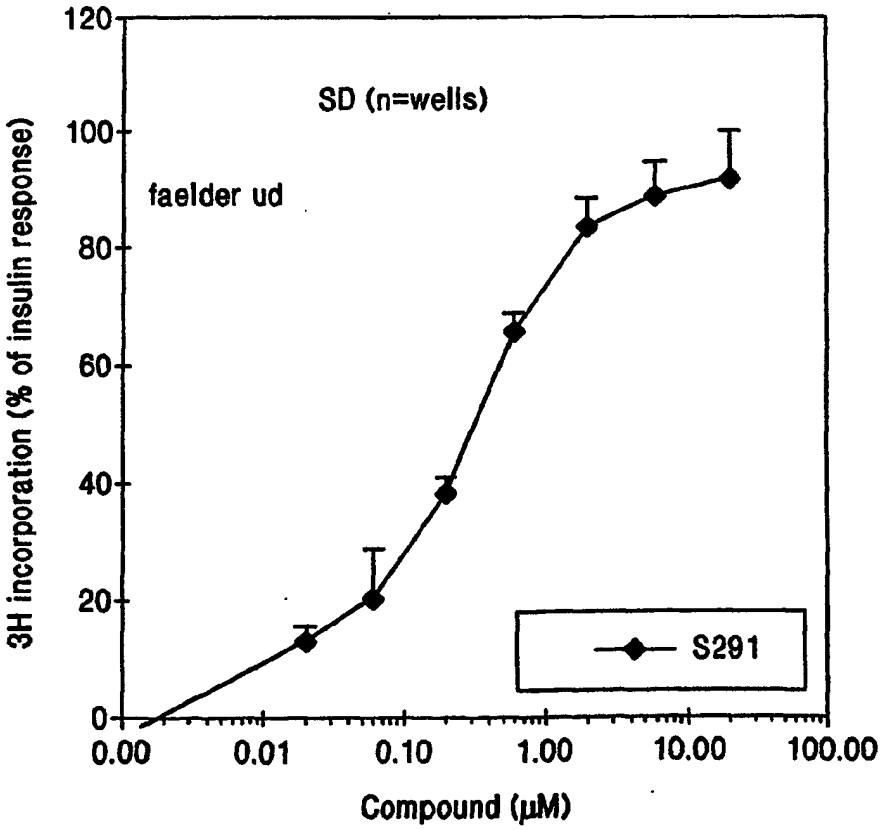


FIG. 71B3

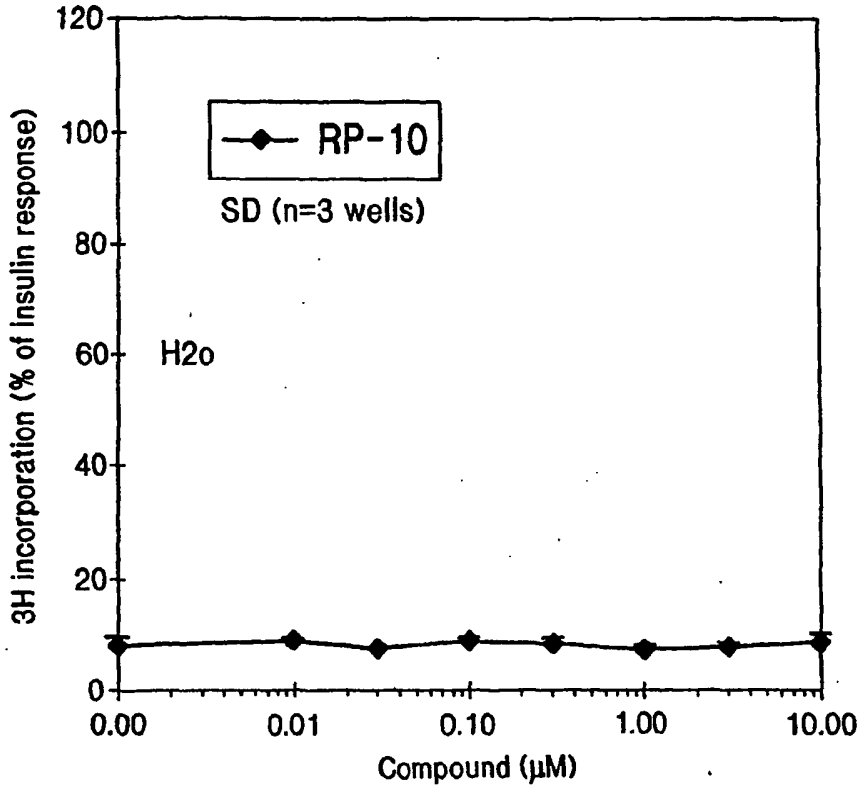


FIG. 71Z2

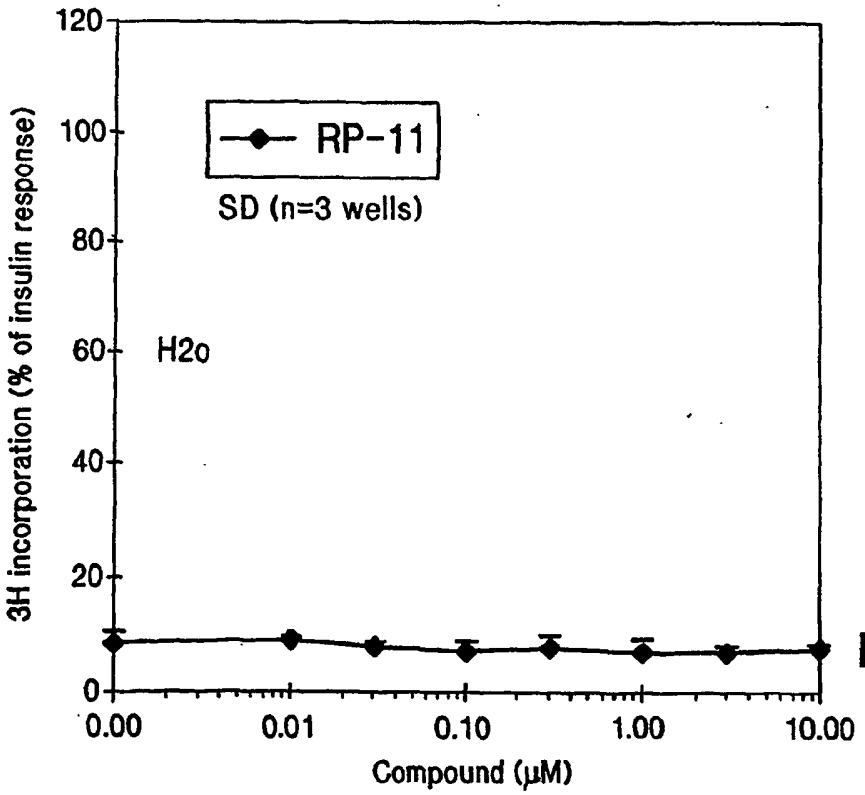


FIG. 71A3

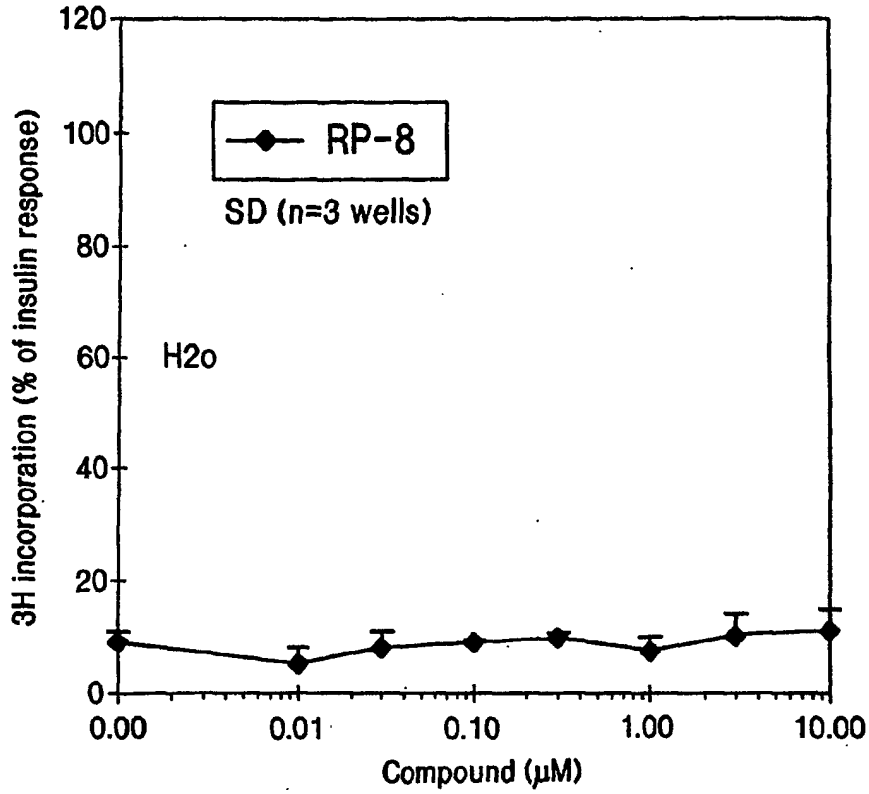


FIG. 71X2

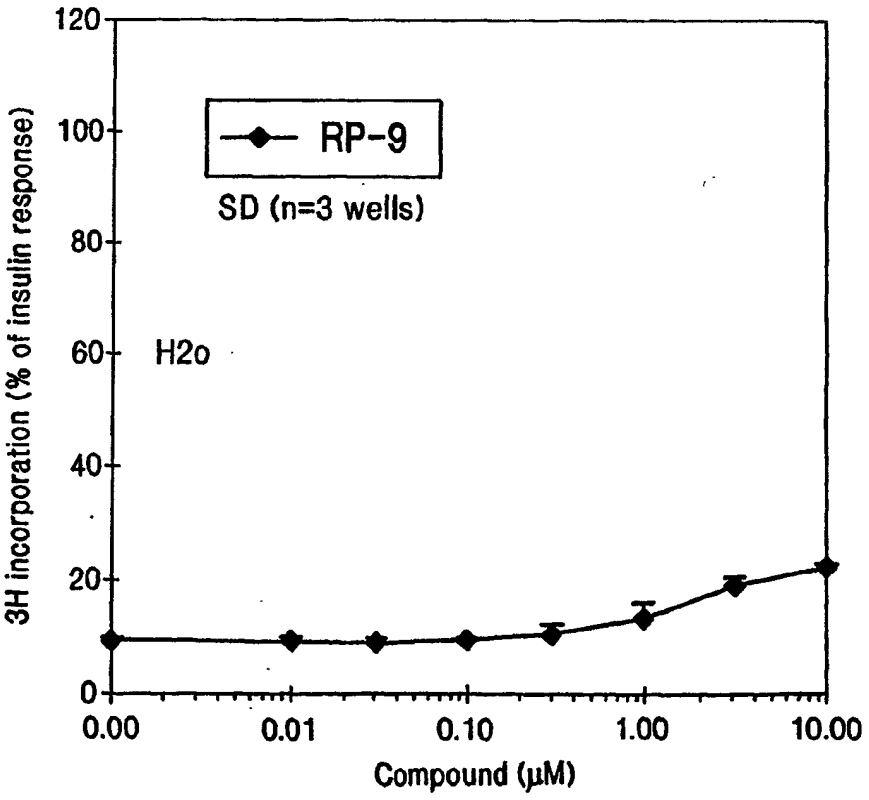


FIG. 71Y2

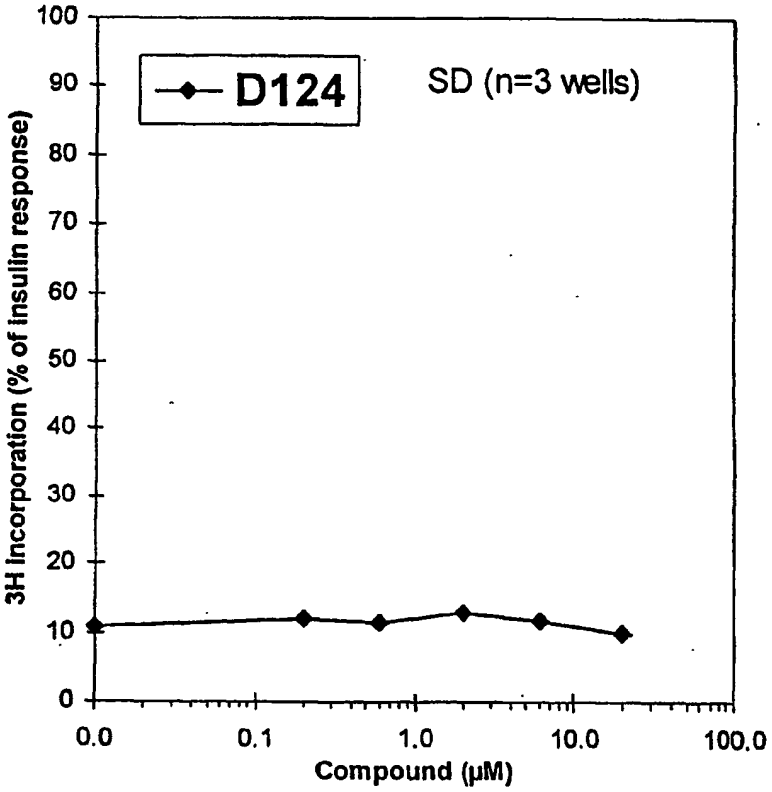

D124
SD (n=3 wells)
3H Incorporation (% of insulin response)
100
90
80
70
60
50
40
30
20
10
0
0.0
0.1
1.0
10.0
100.0
Compound (μM)

FIG. 71W2

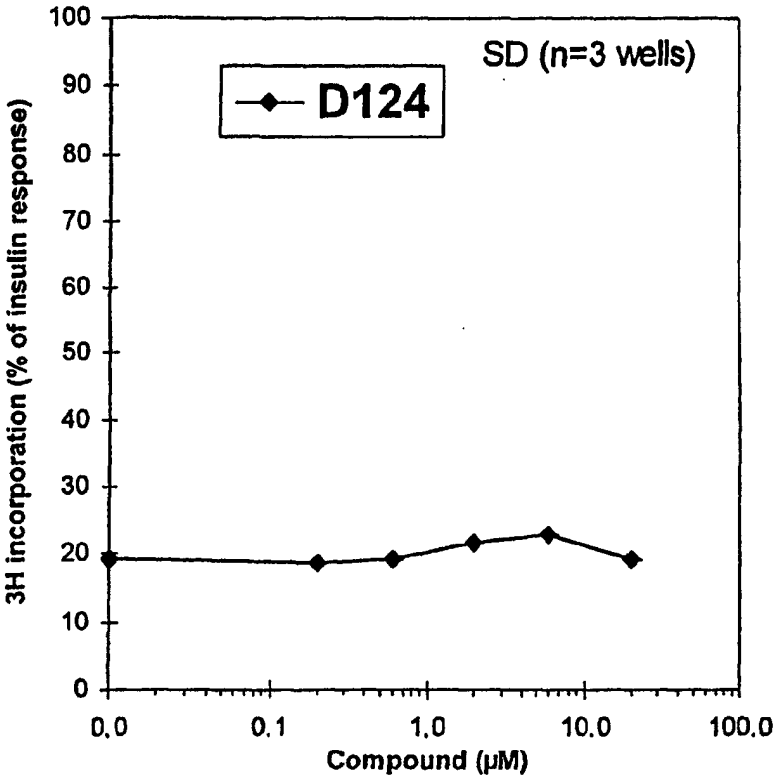


FIG. 71U2

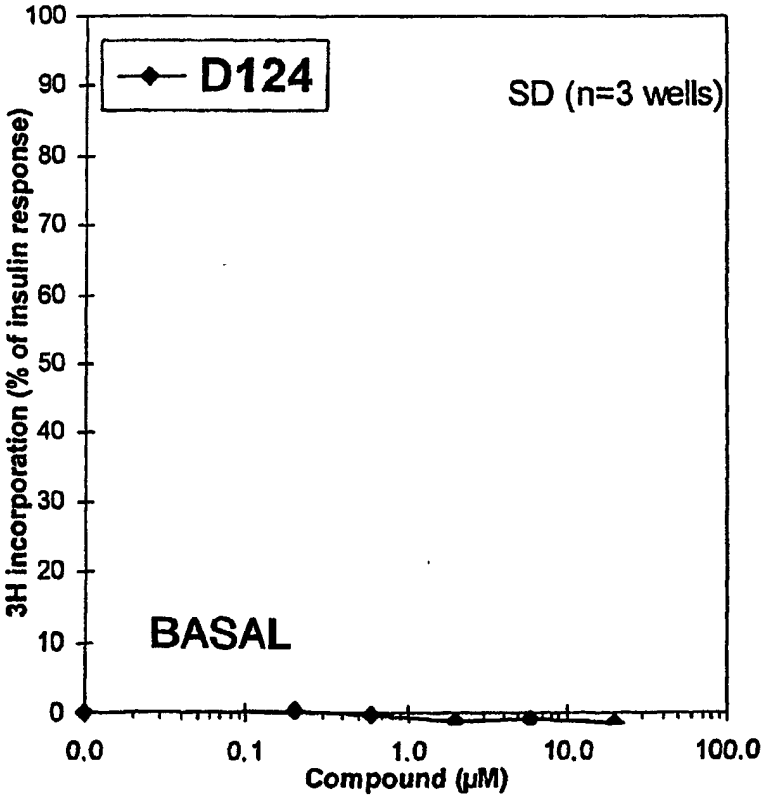


FIG. 71V2

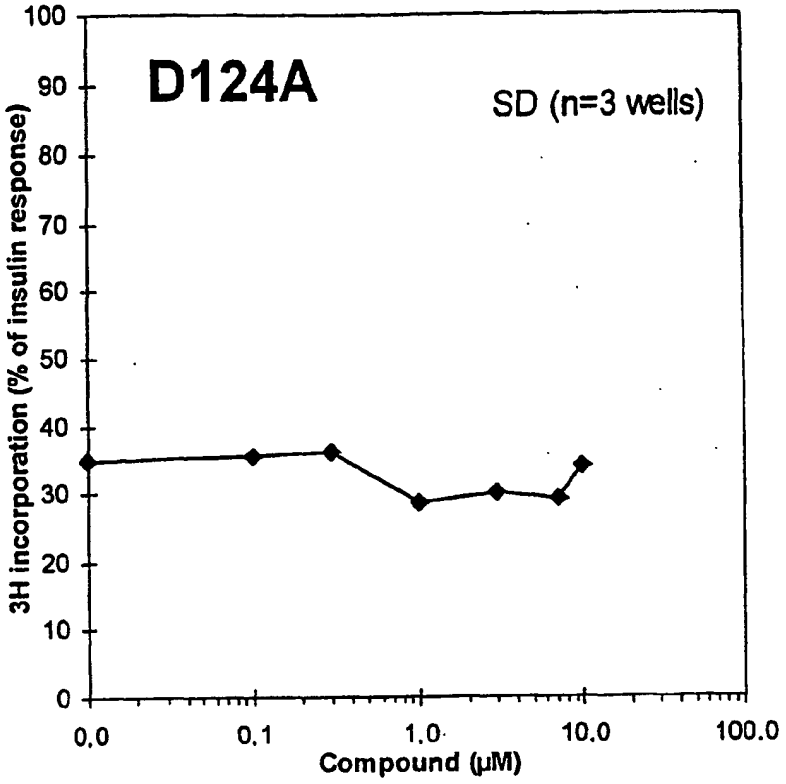


FIG. 71S2

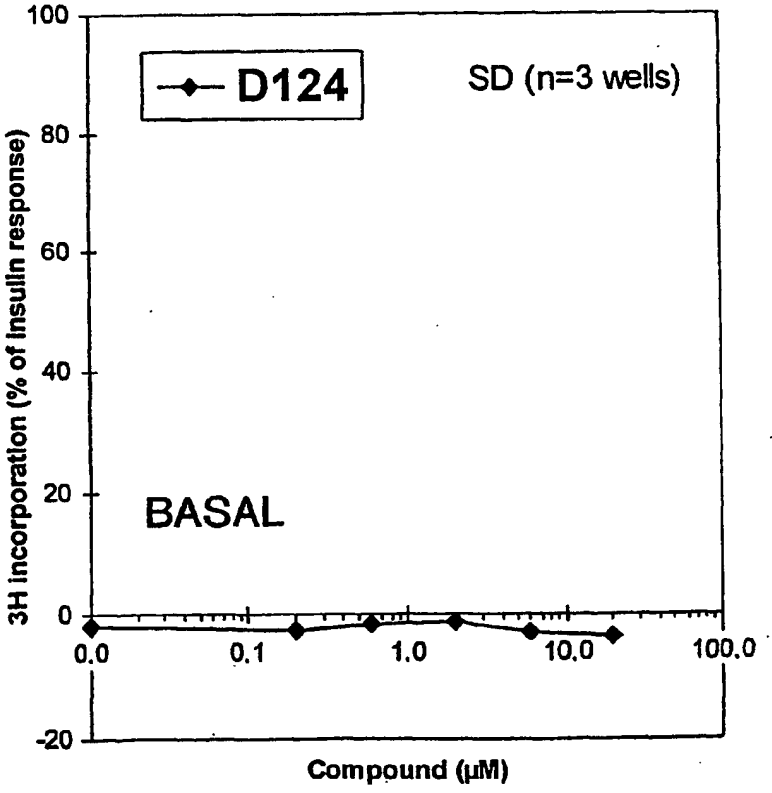


FIG. 71T2

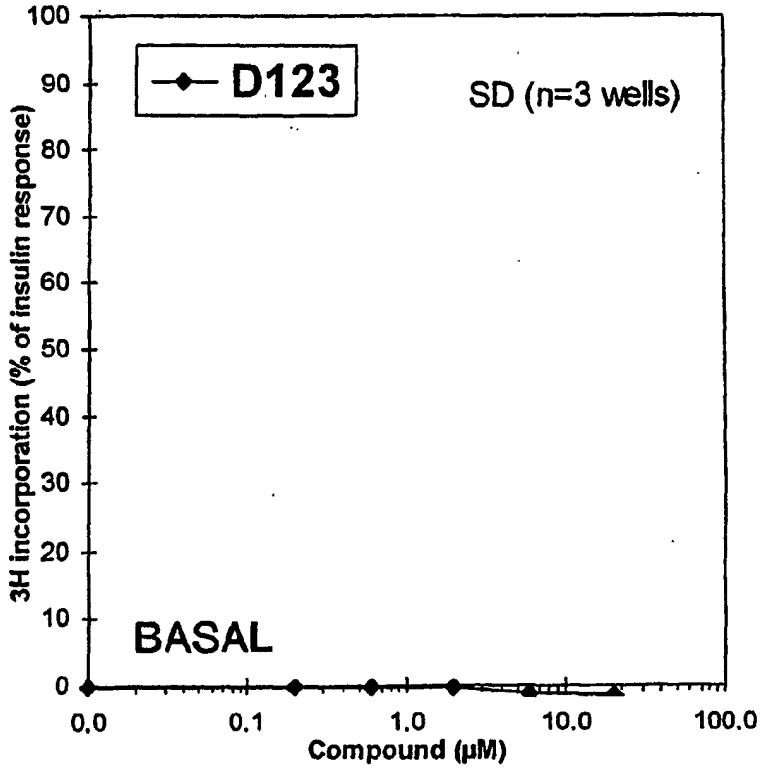


FIG. 71Q2

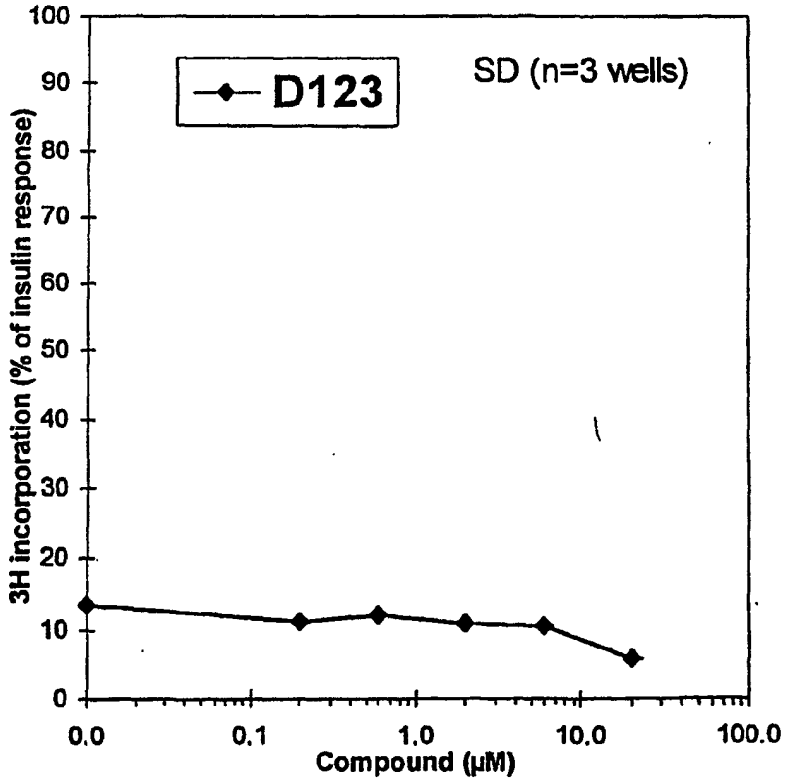


FIG. 71R2

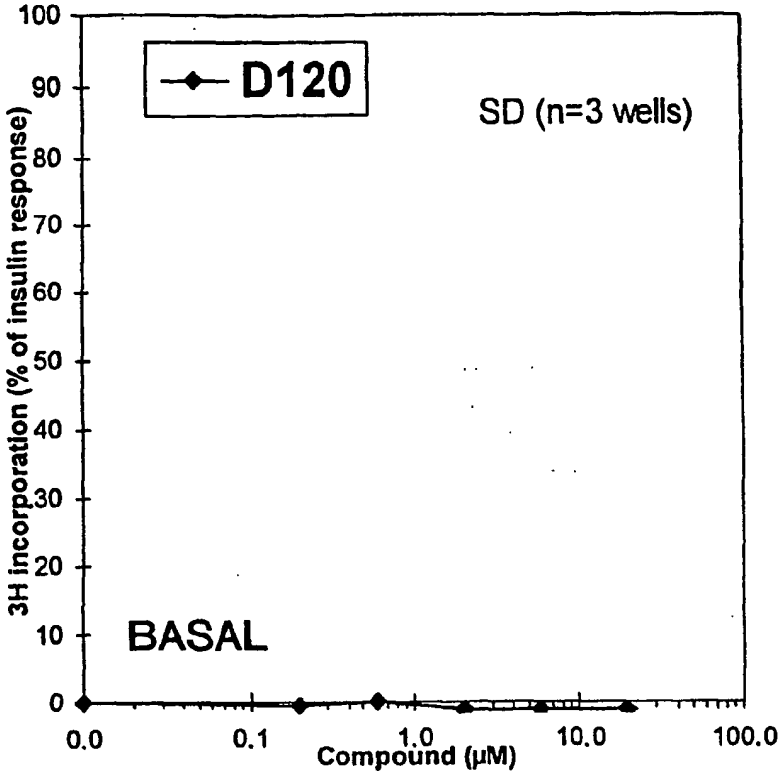


FIG. 71O2

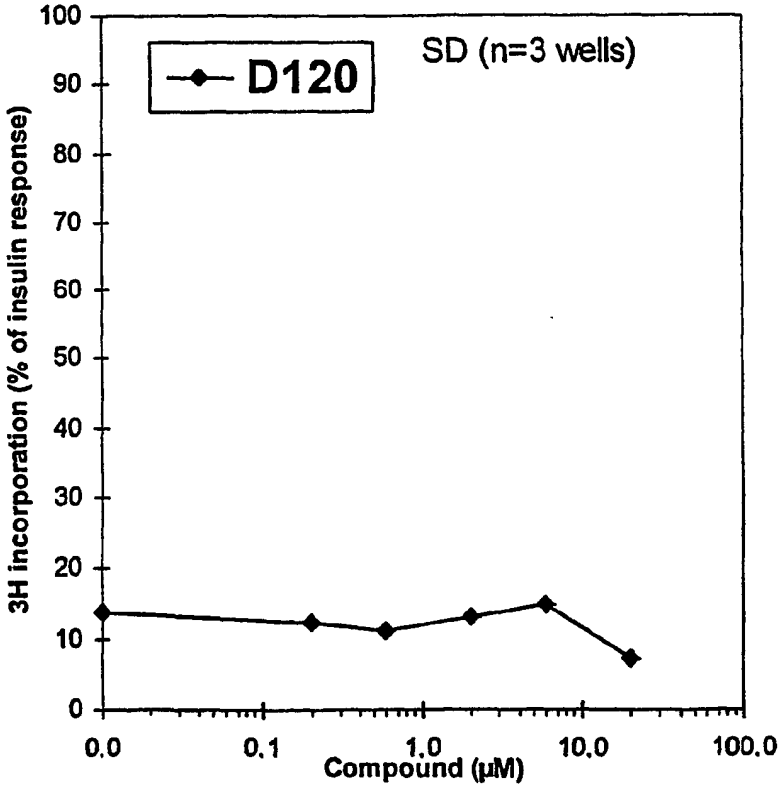


FIG. 71P2

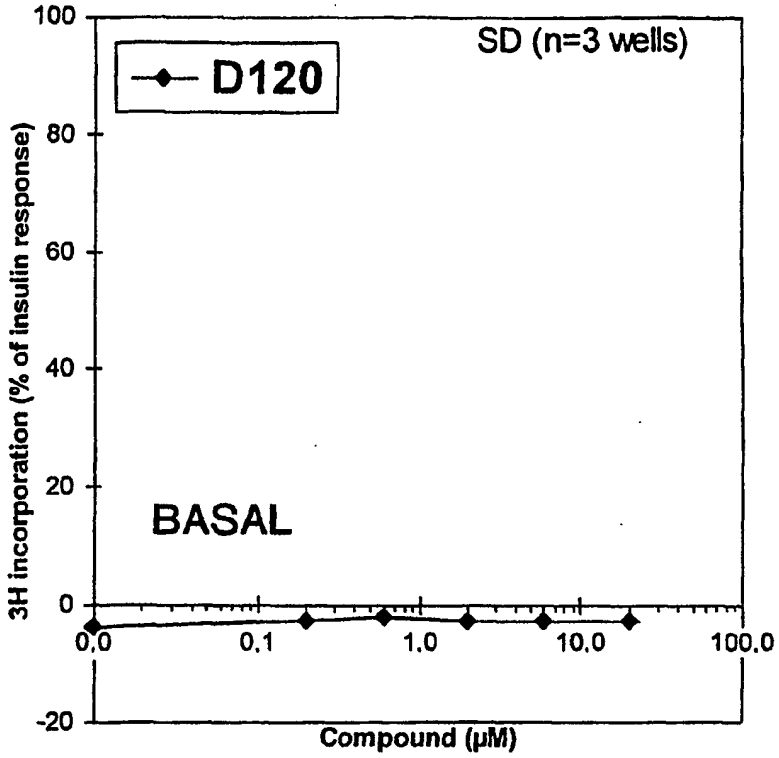


FIG. 71M2

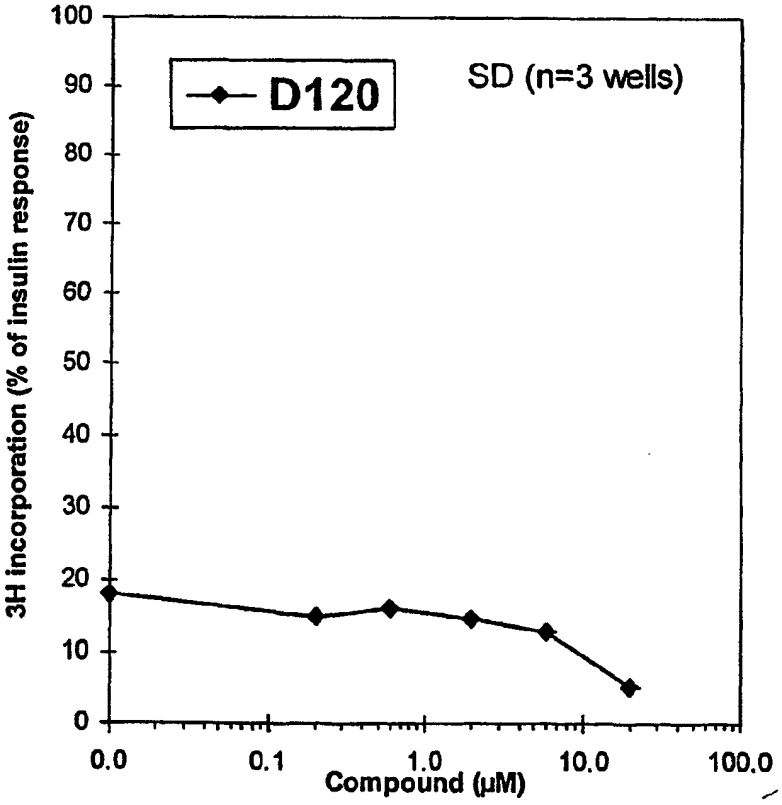


FIG. 71N2

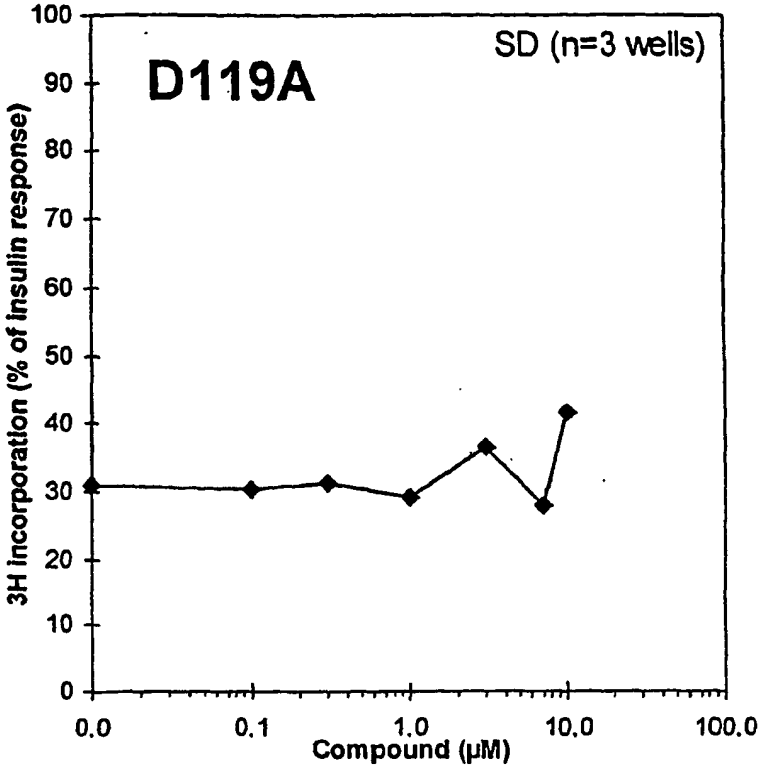


FIG. 71K2

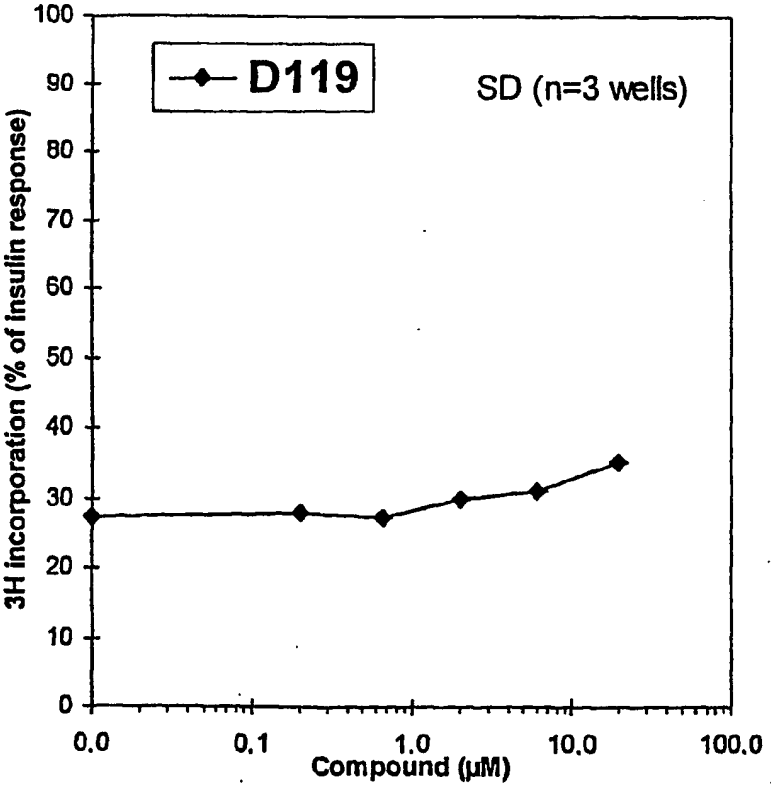


FIG. 71L2

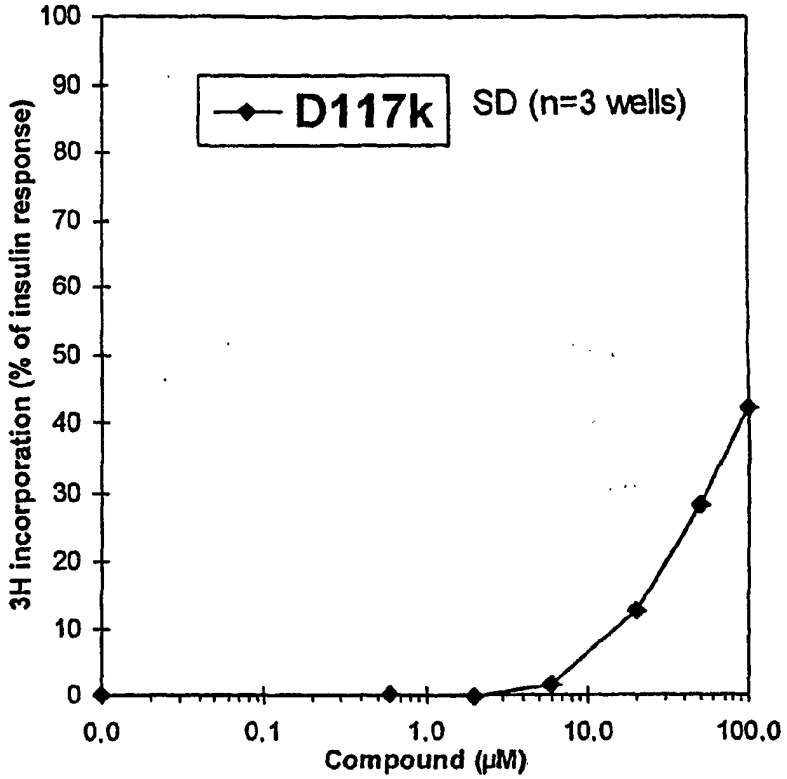


FIG. 71I2

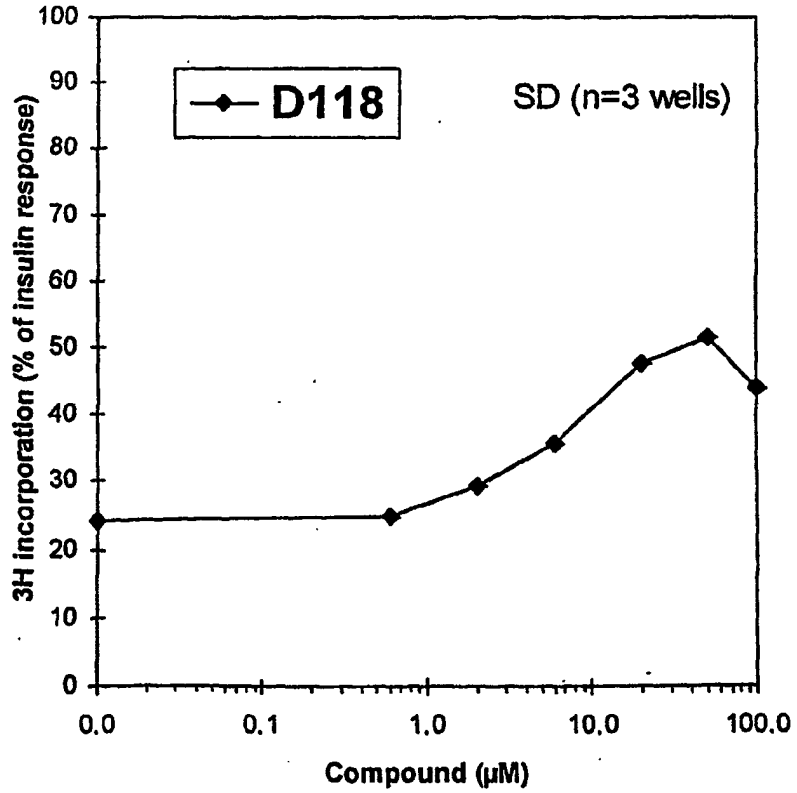


FIG. 71J2

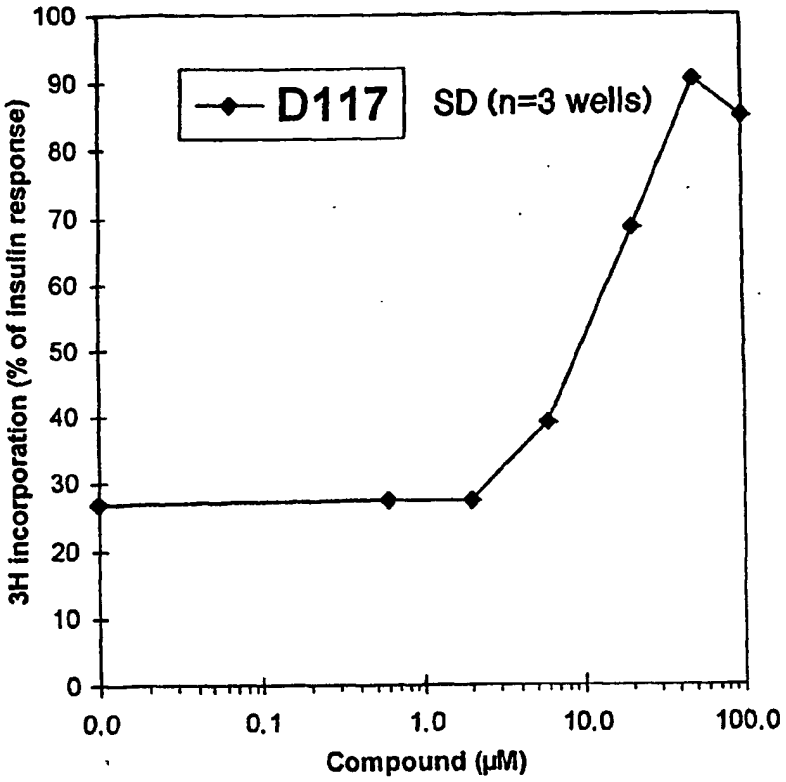


FIG. 71G2

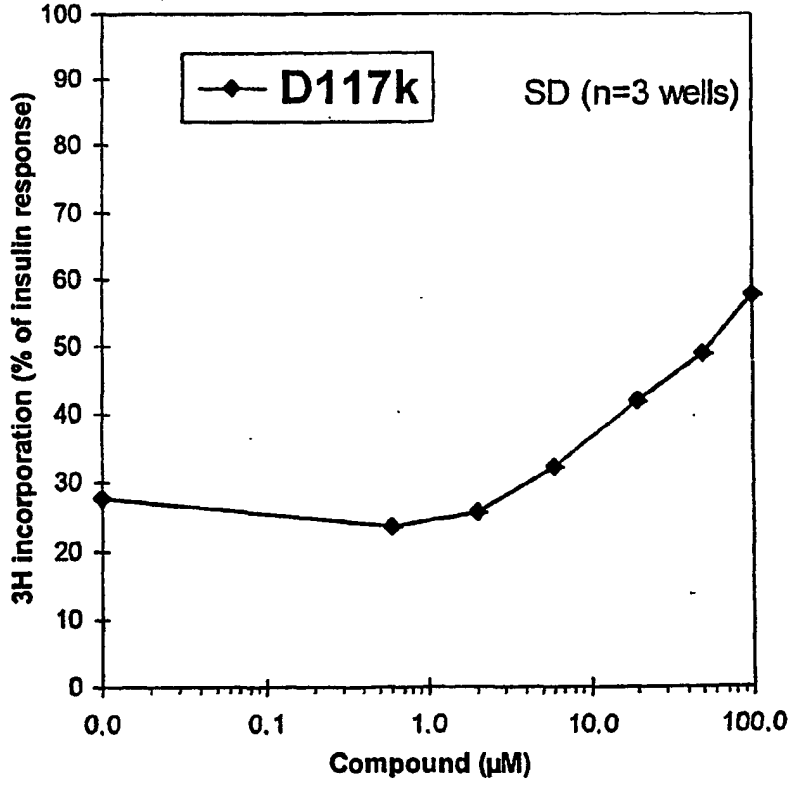


FIG. 71H2

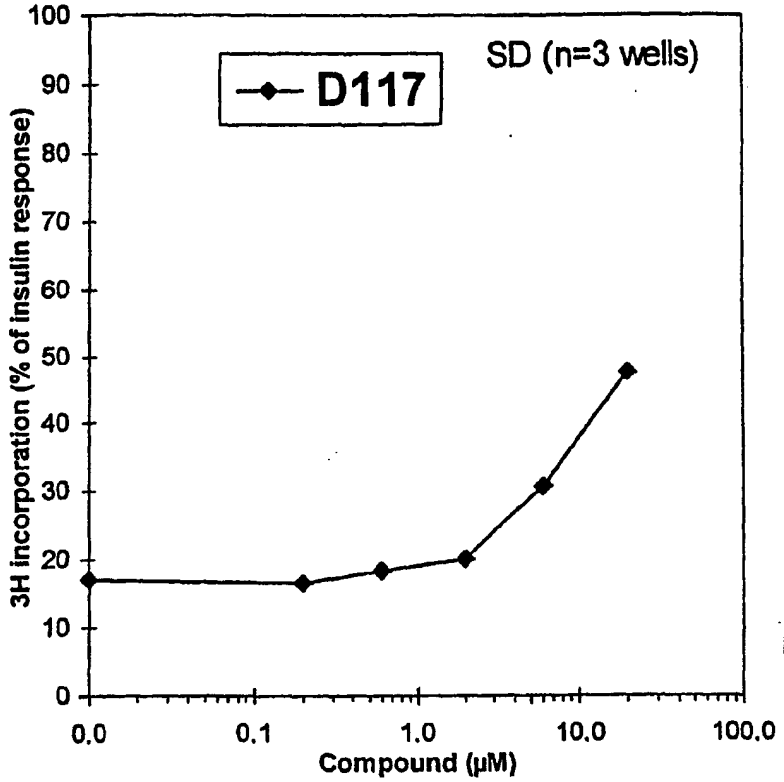


FIG. 71E2

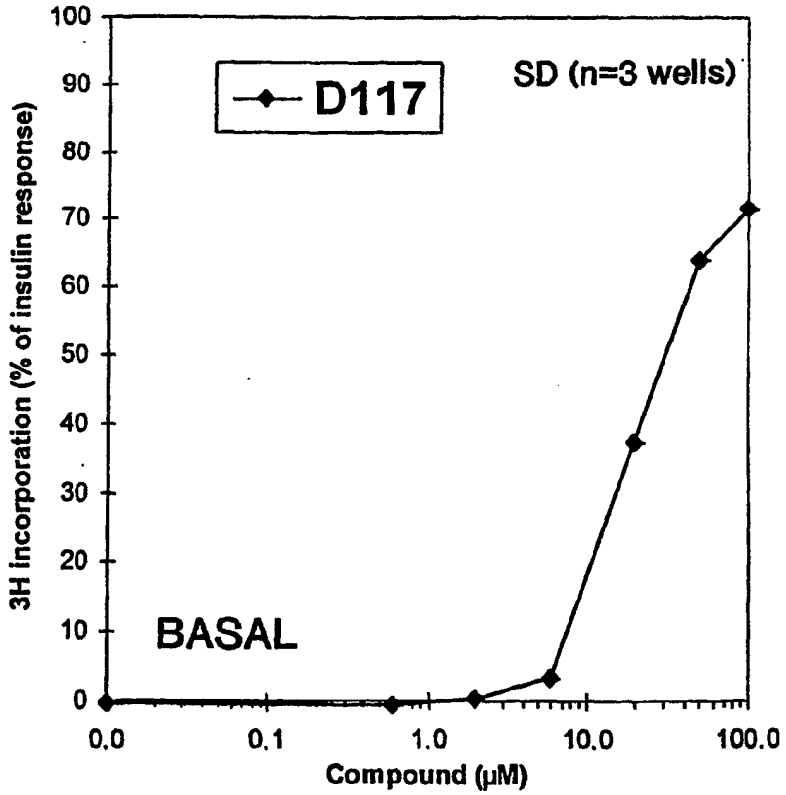


FIG. 71F2

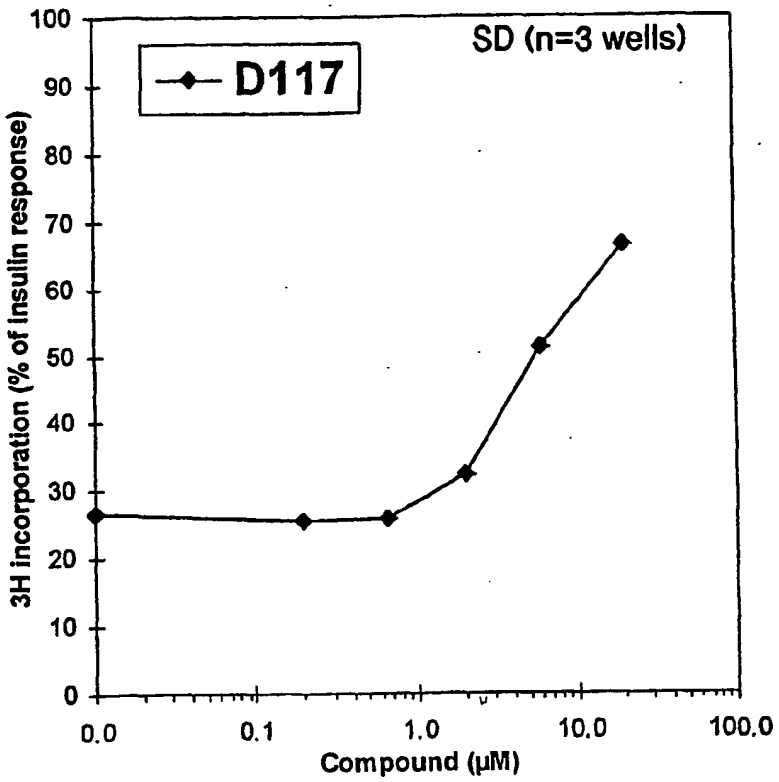


FIG. 71C2

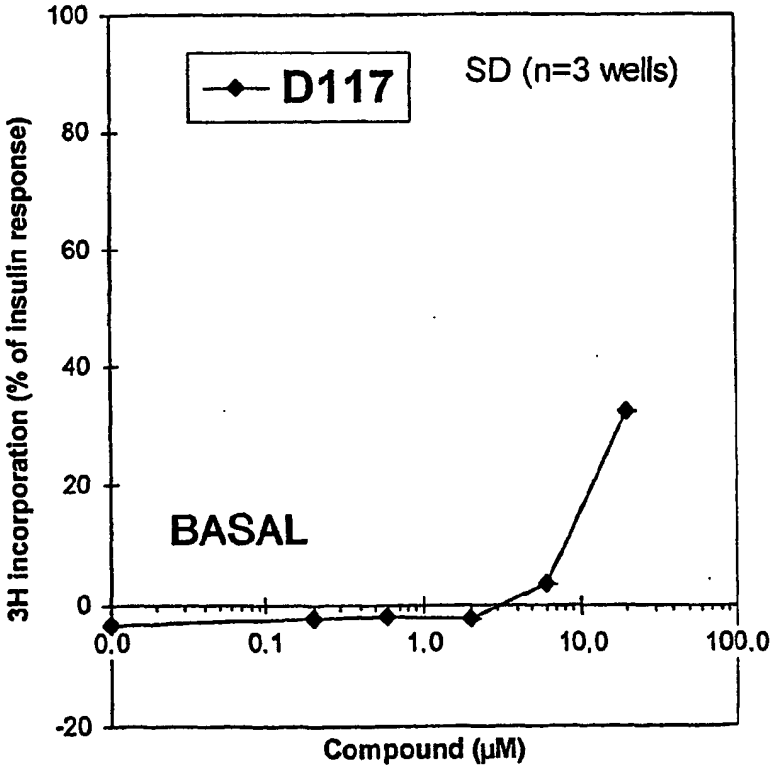


FIG. 71D2

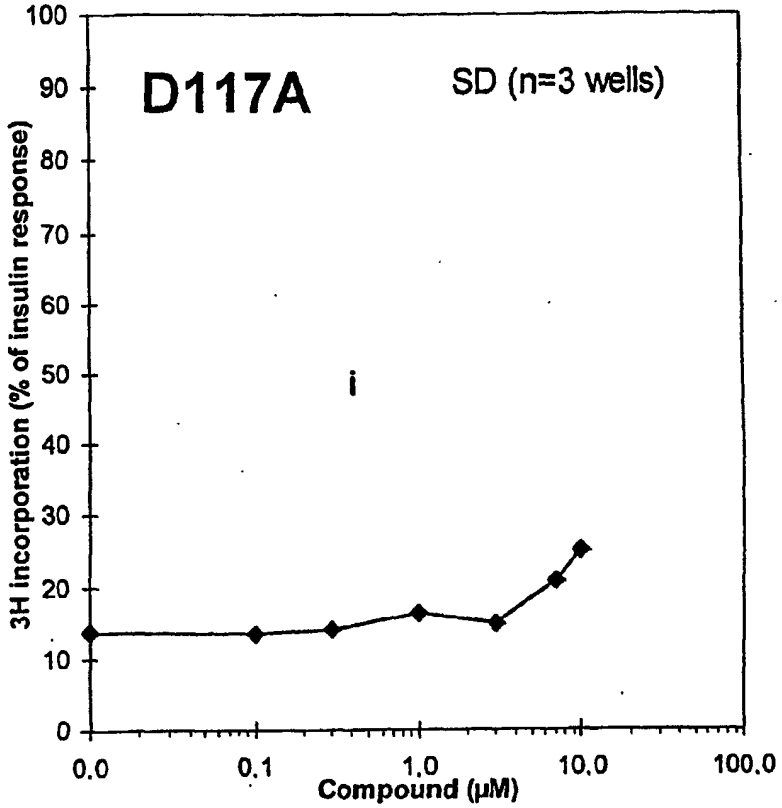


FIG. 71A2

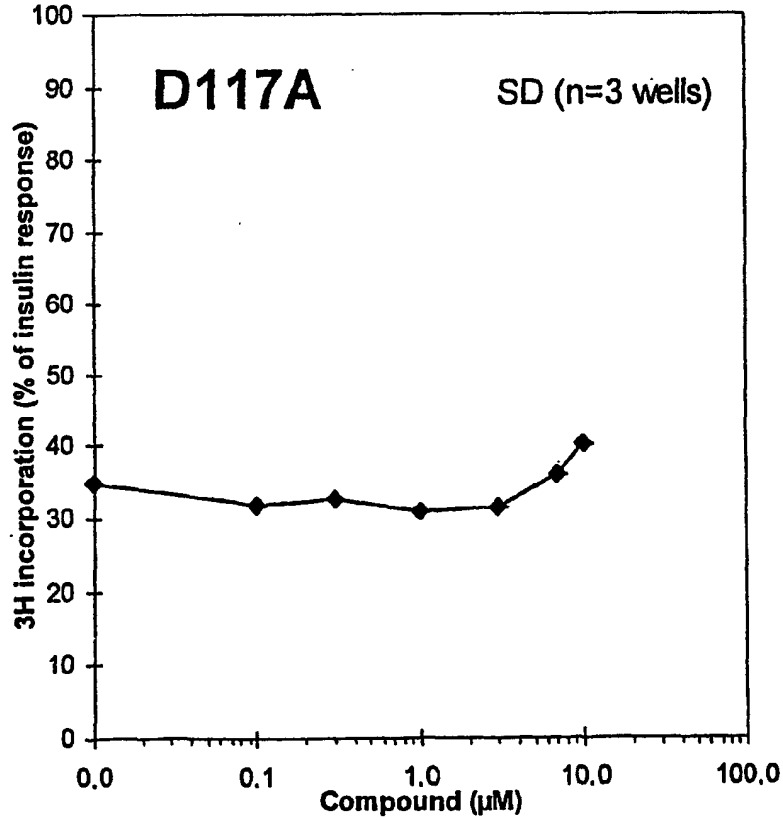


FIG. 71B2

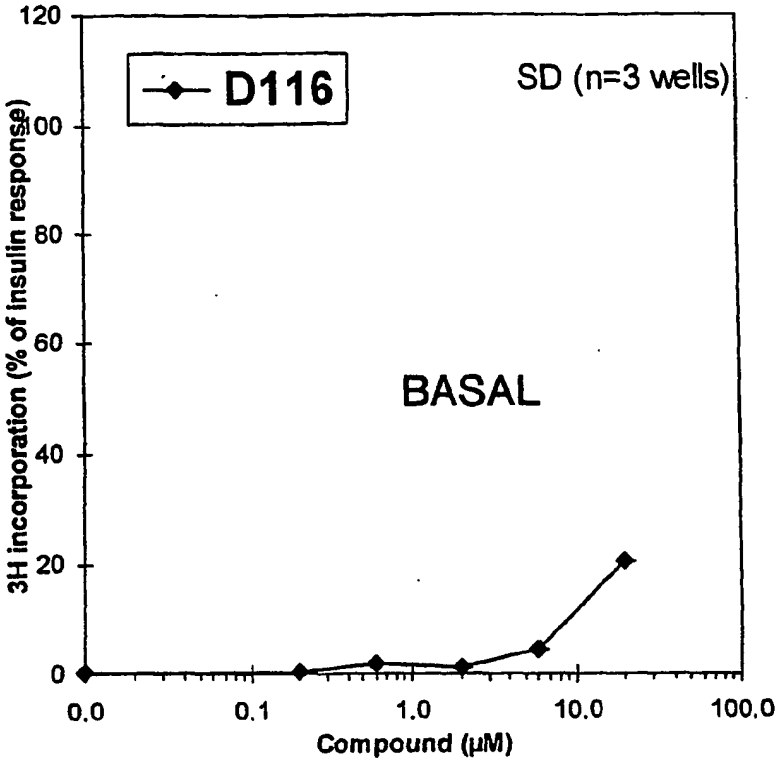


FIG. 71Y

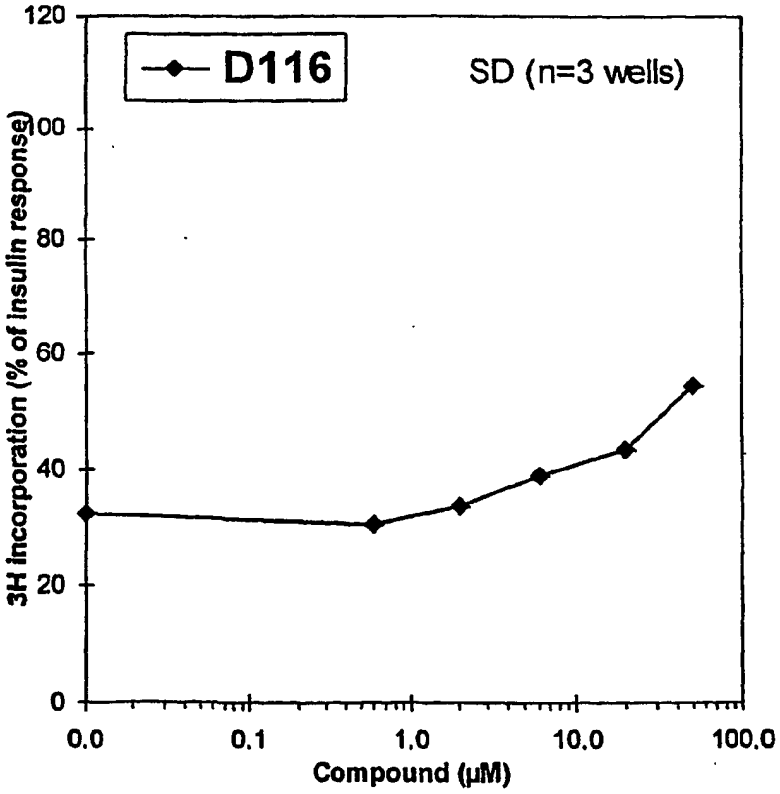


FIG. 71Z

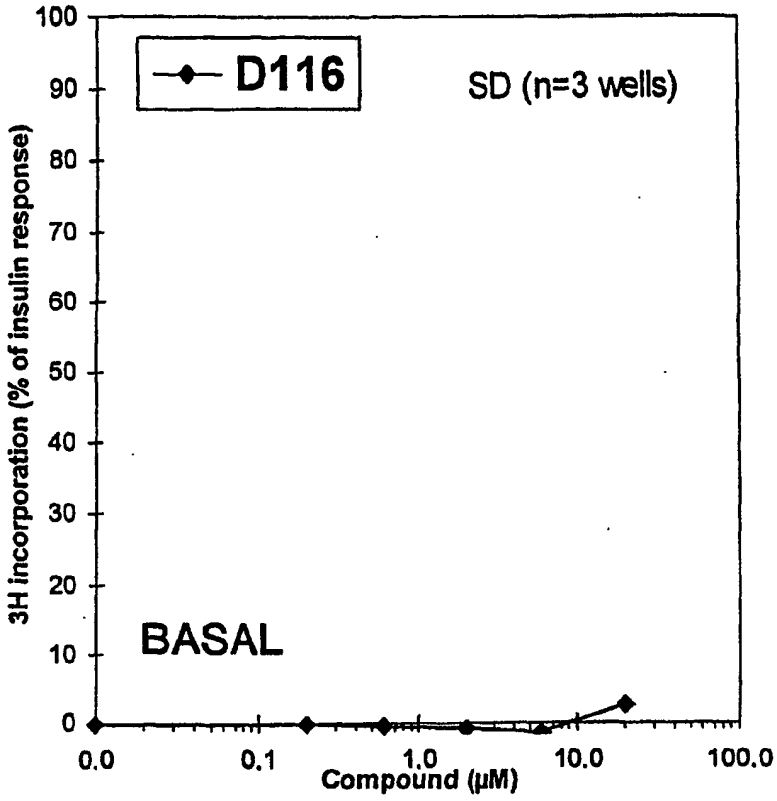


FIG. 71W

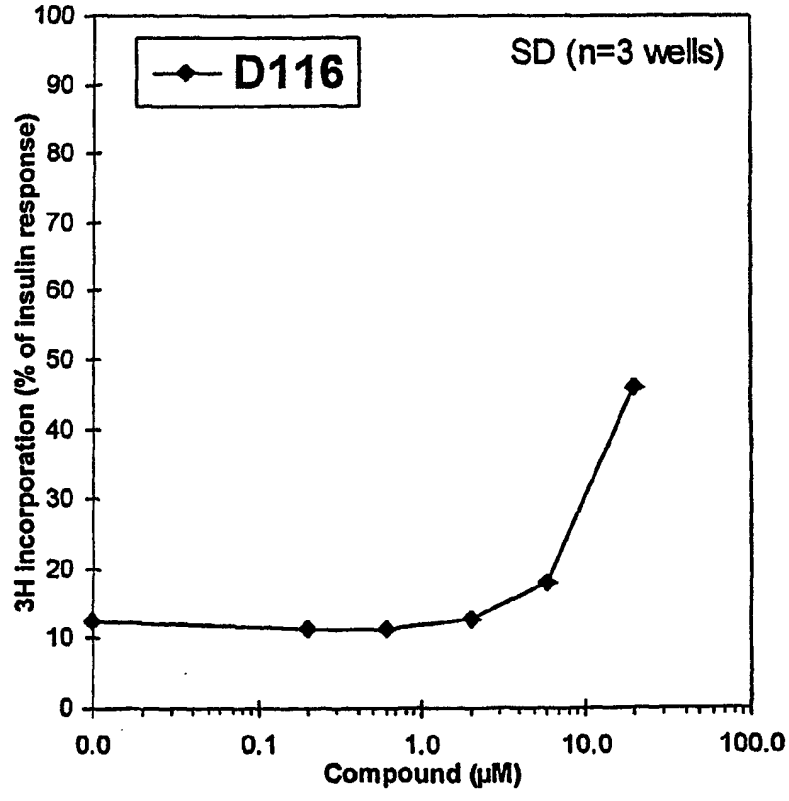


FIG. 71X

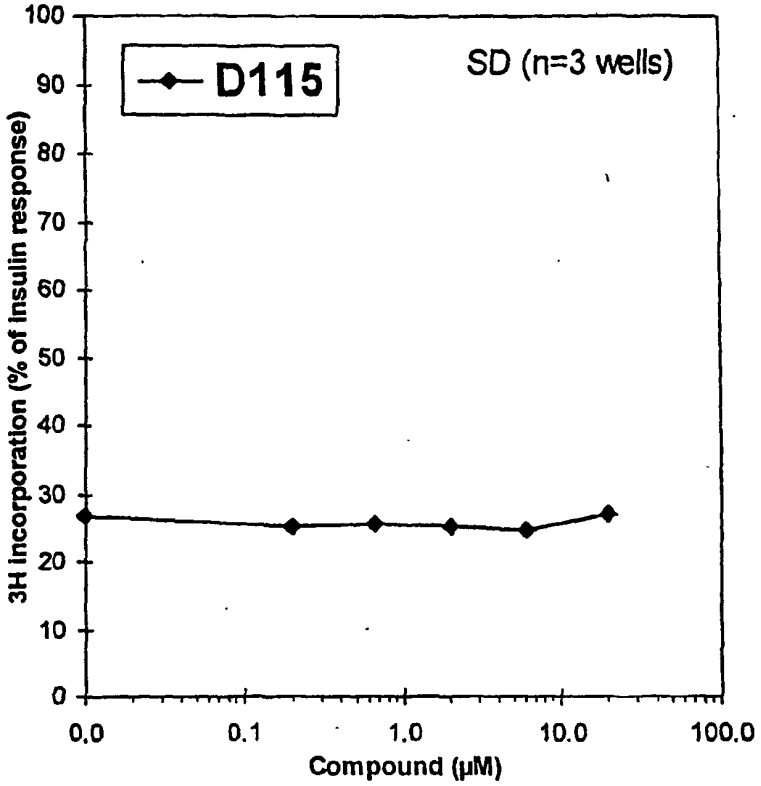


FIG. 71U

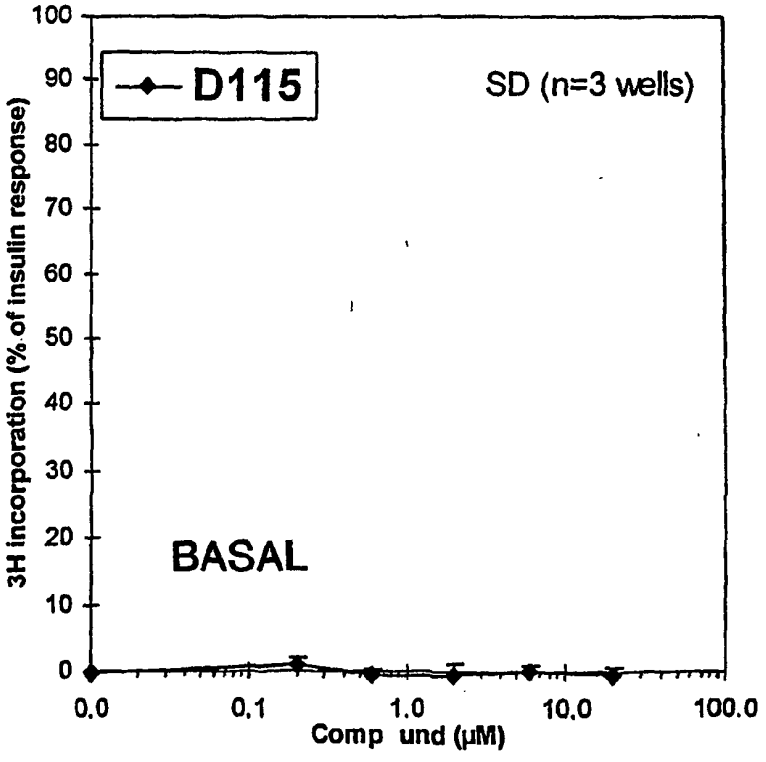


FIG. 71V

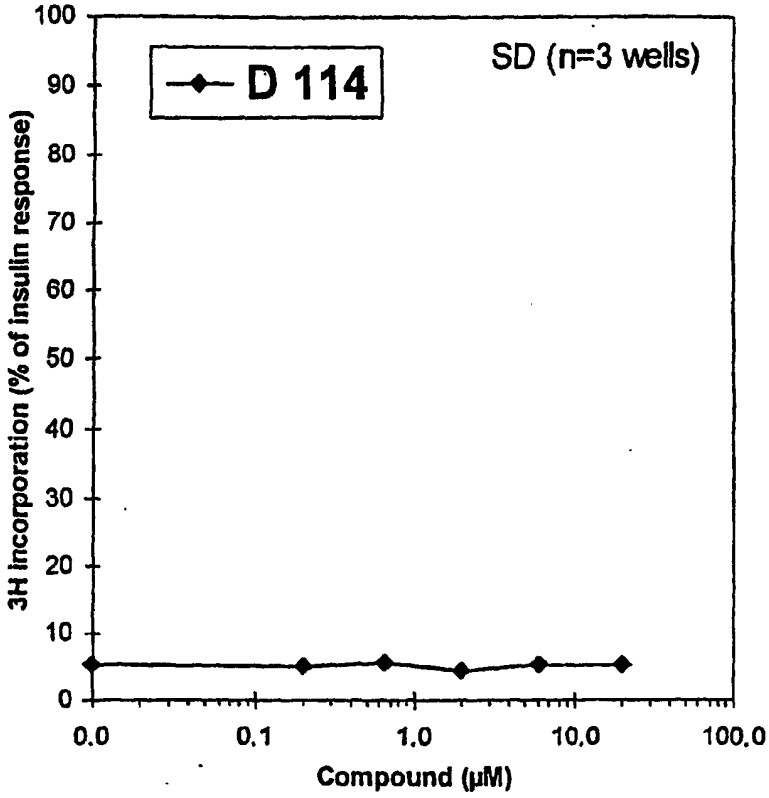


FIG. 71S

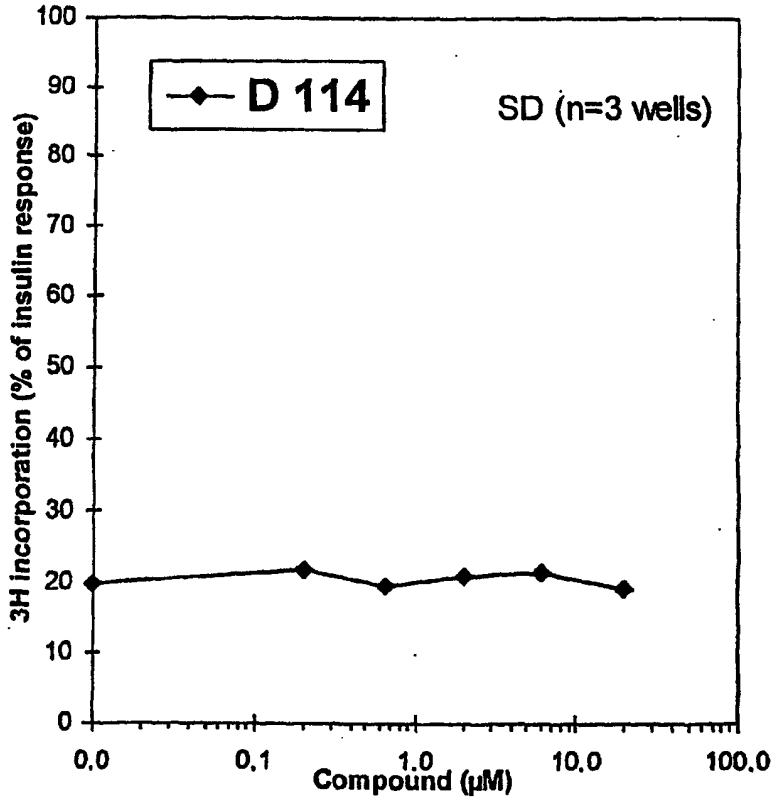


FIG. 71T

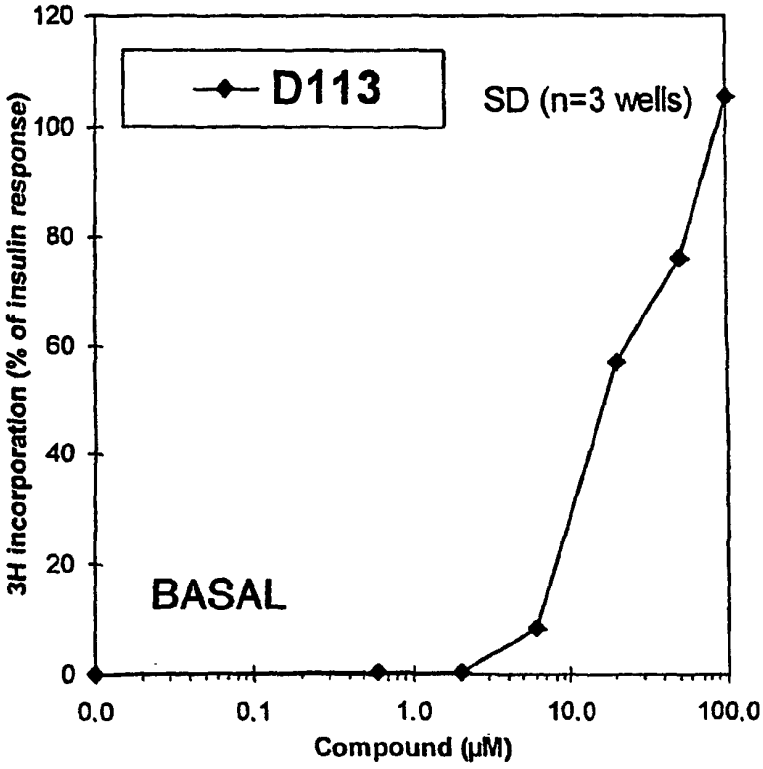


FIG. 71Q

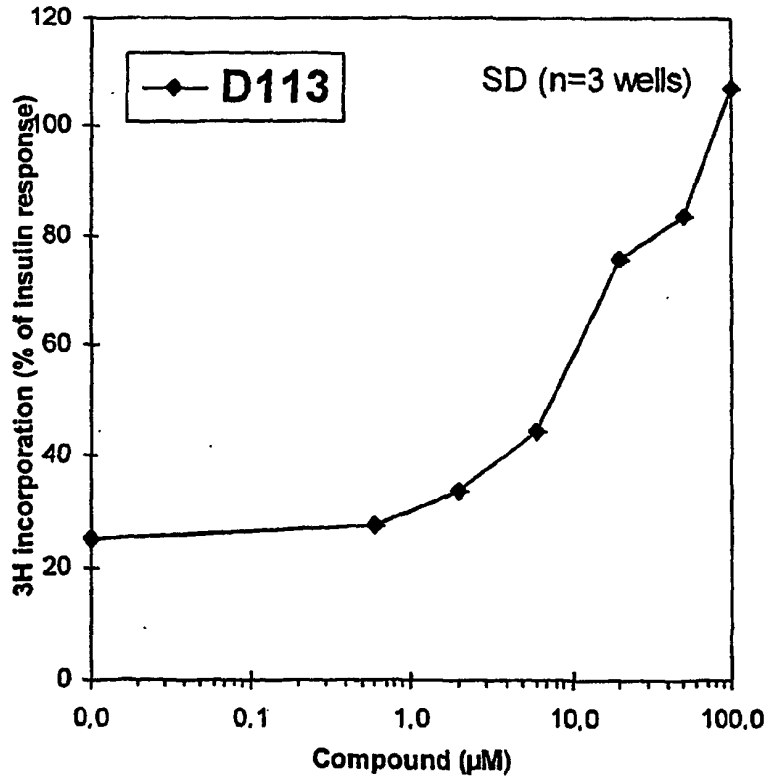


FIG. 71R

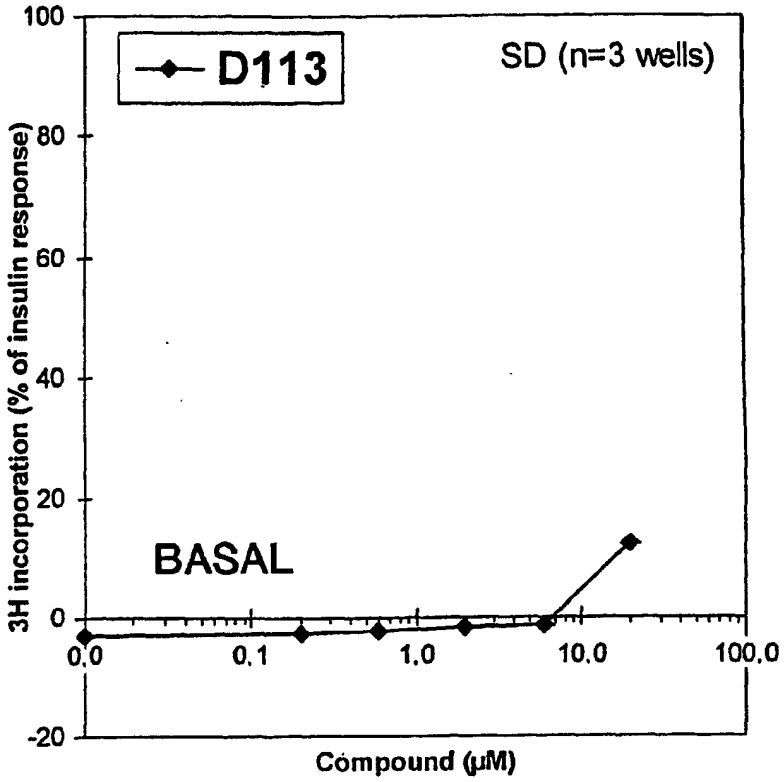


FIG. 71O

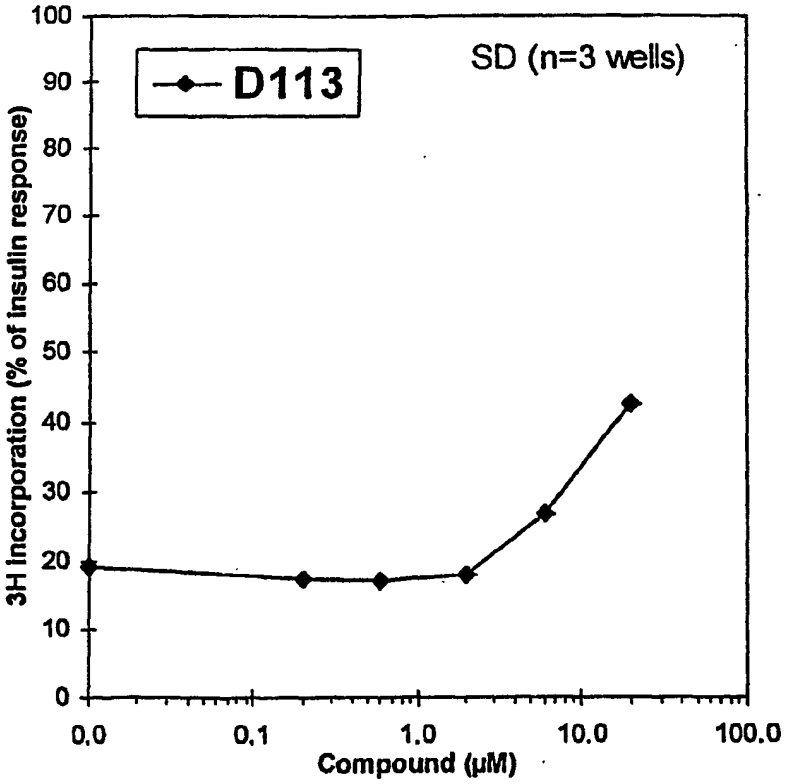


FIG. 71P

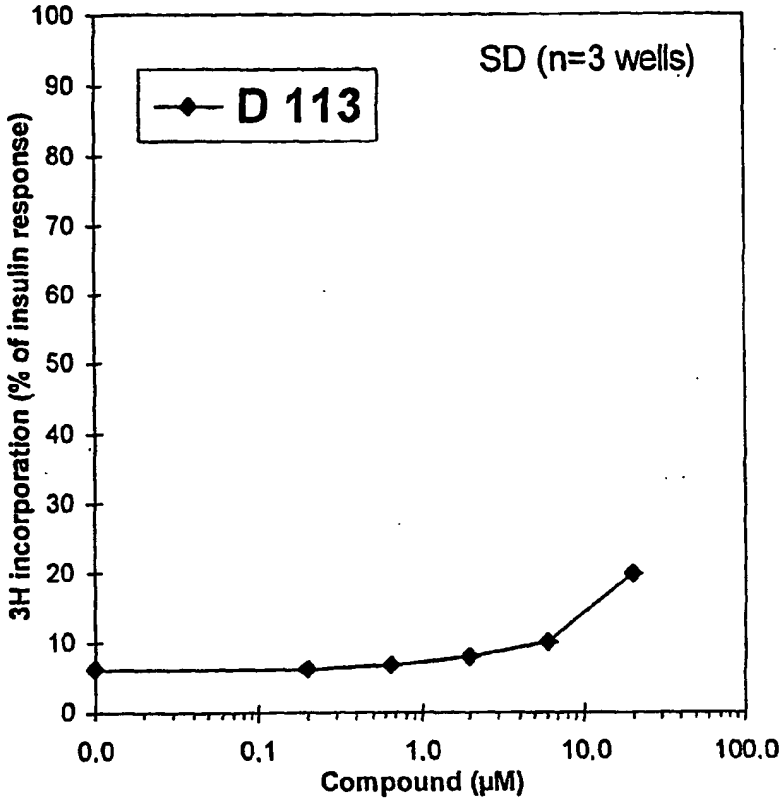


FIG. 71M

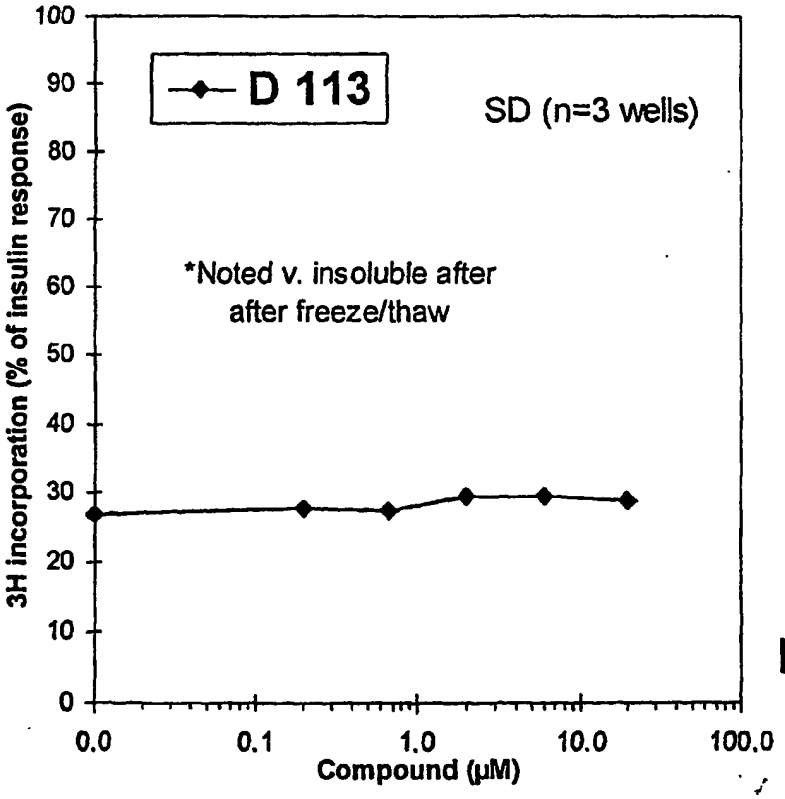


FIG. 71N

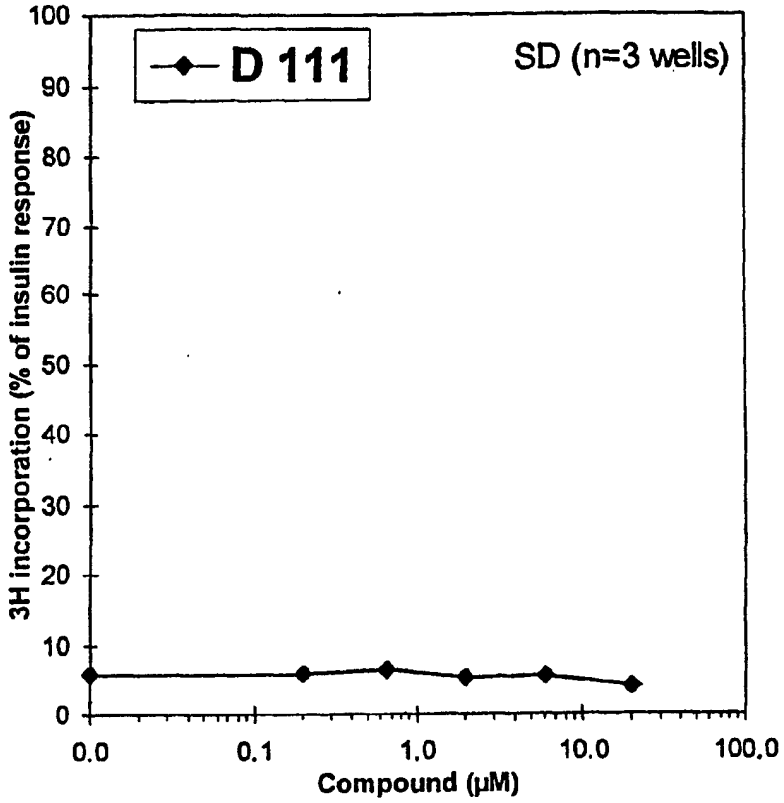


FIG. 71K

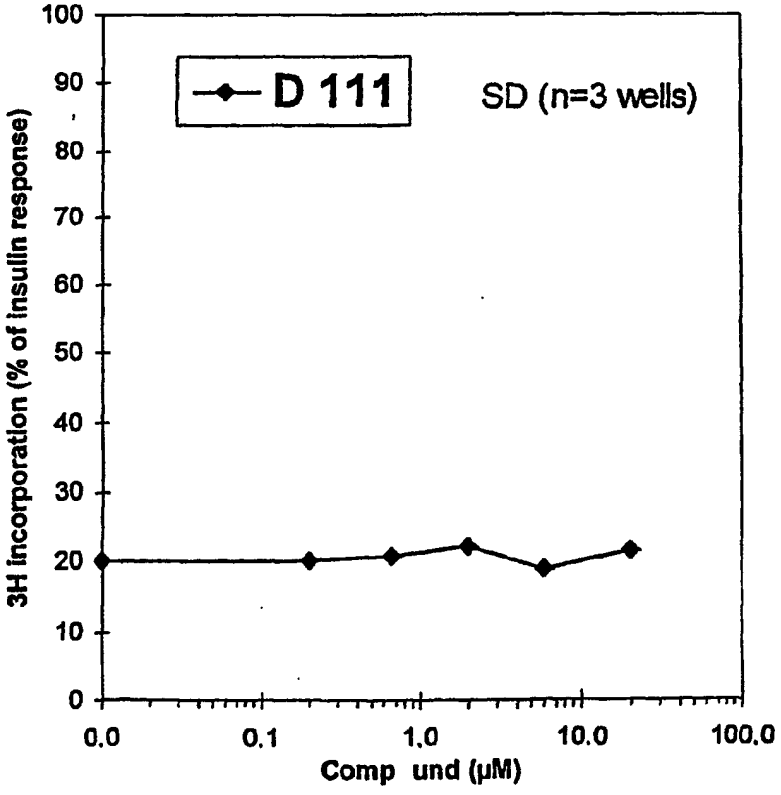


FIG. 71L

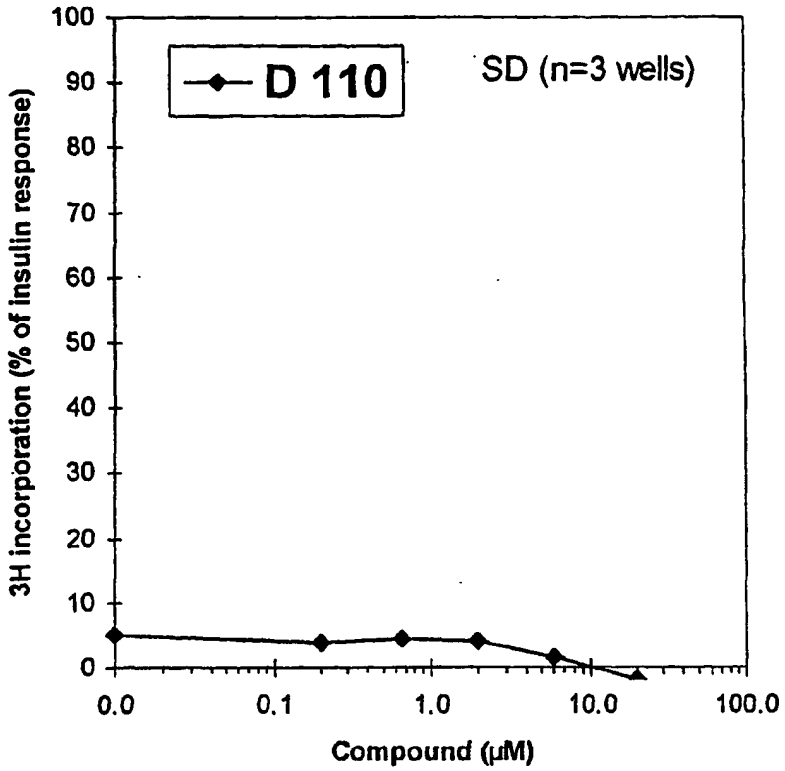


FIG. 71I

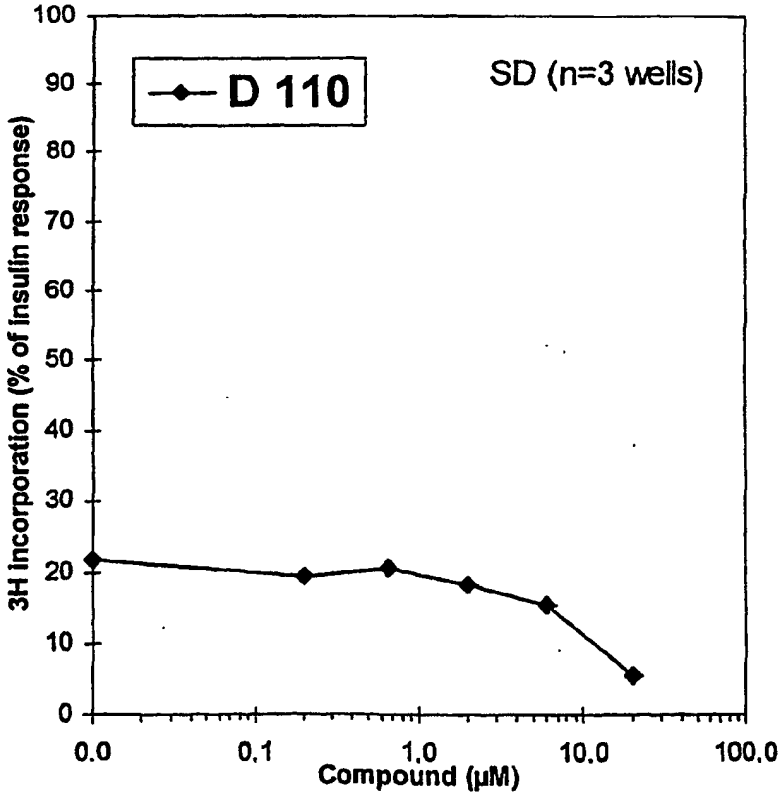


FIG. 71J

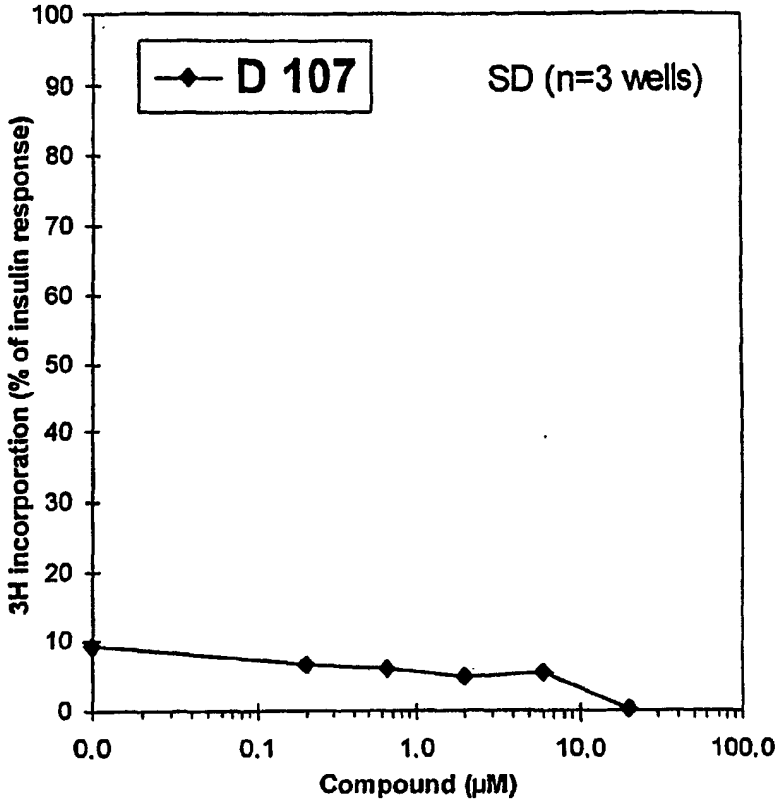


FIG. 71G

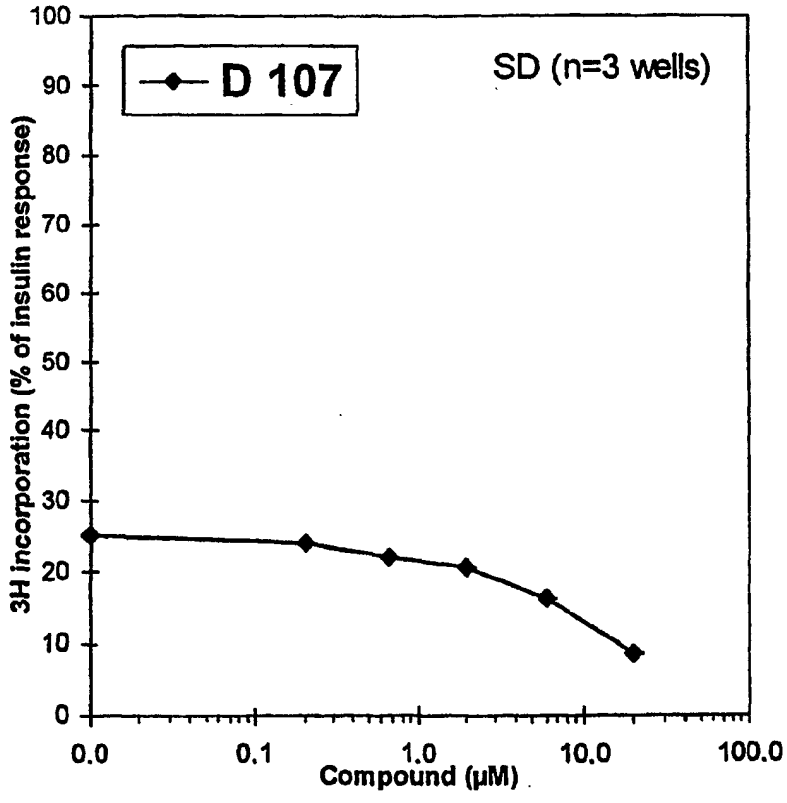


FIG. 71H

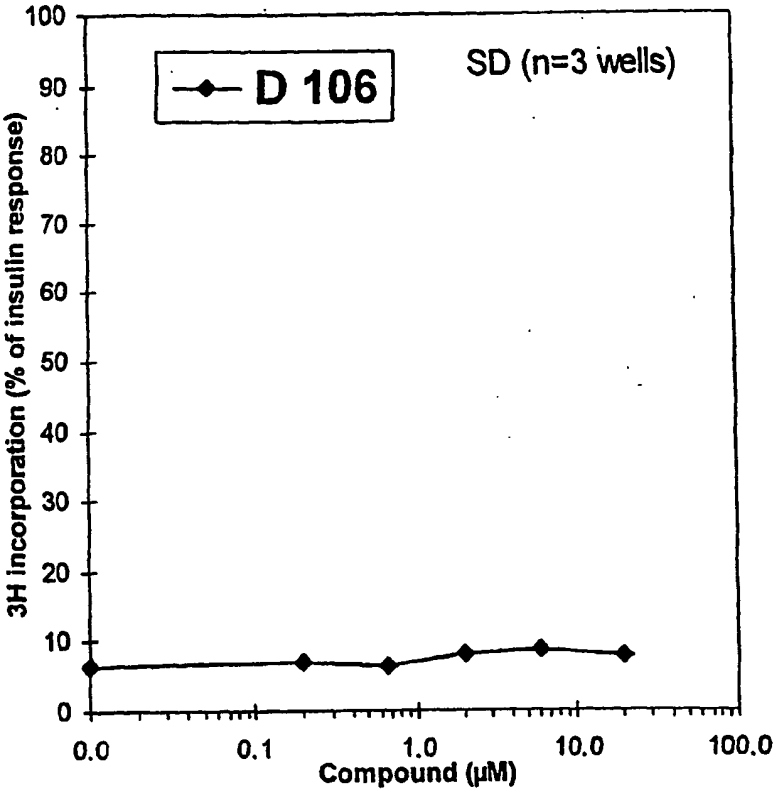


FIG. 71E

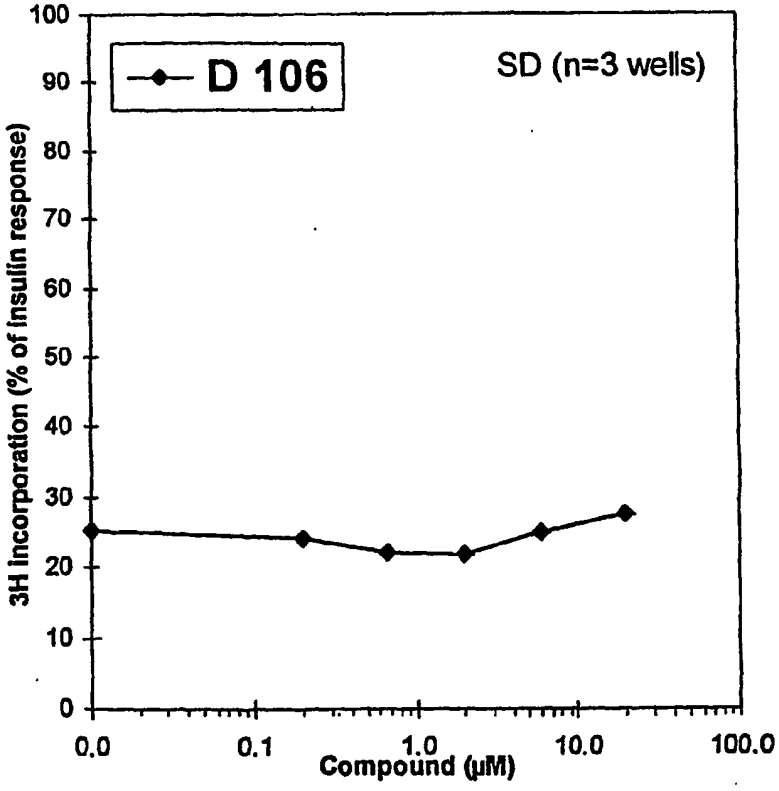


FIG. 71F

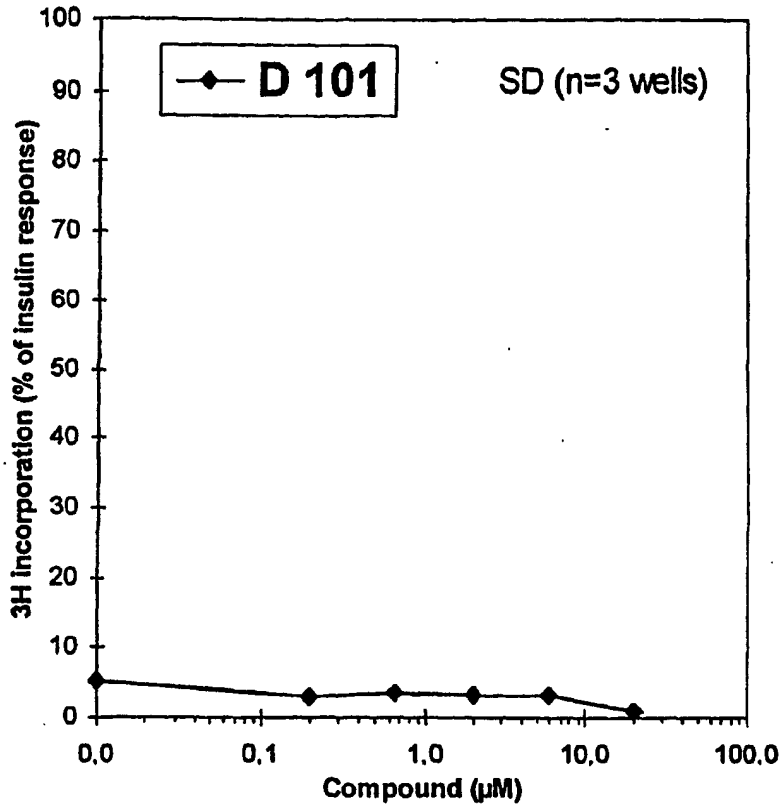


FIG. 71C

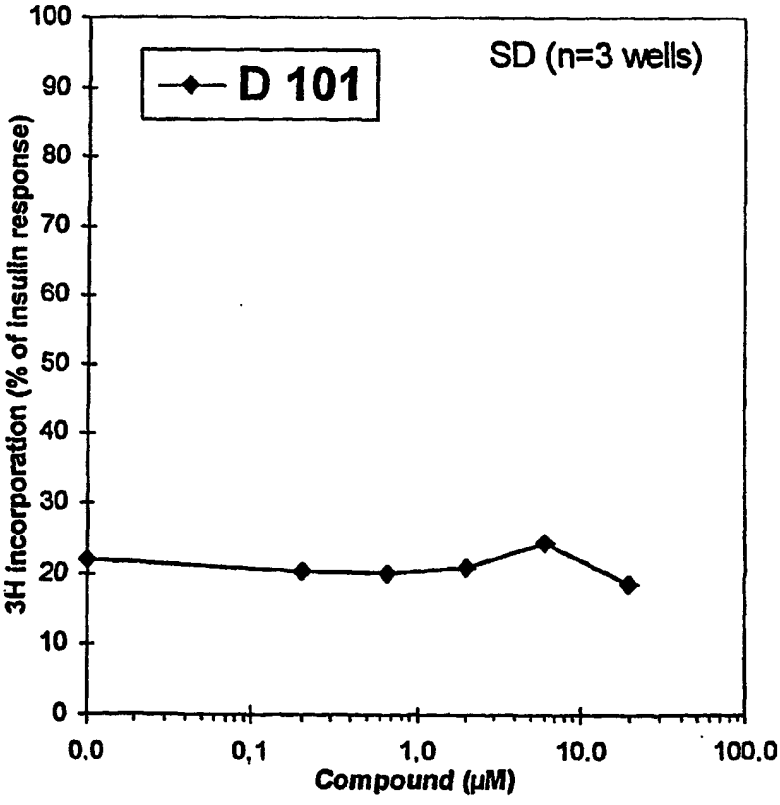


FIG. 71D

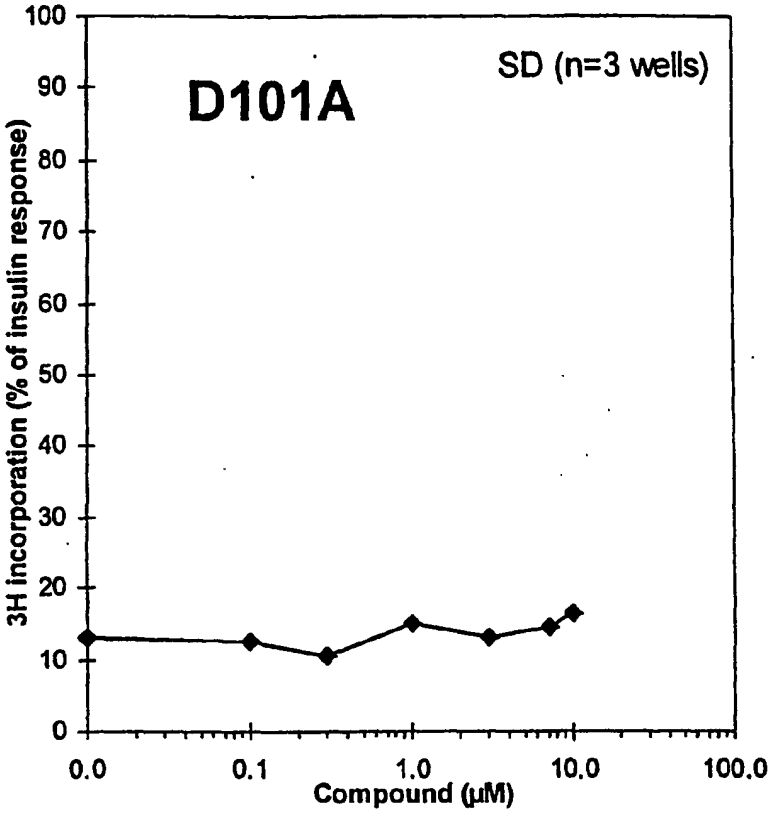


FIG. 71A

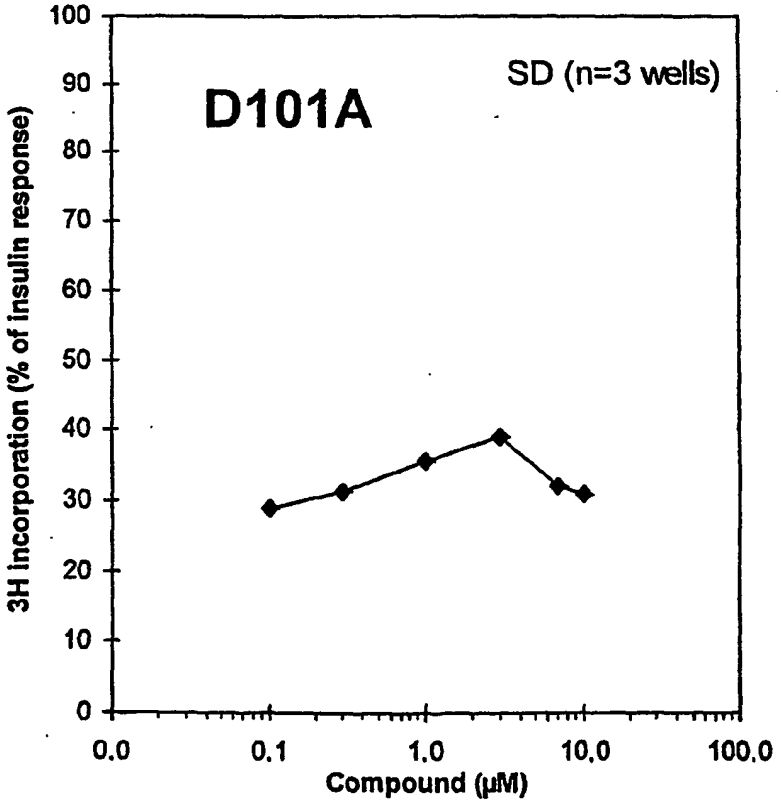


FIG. 71B

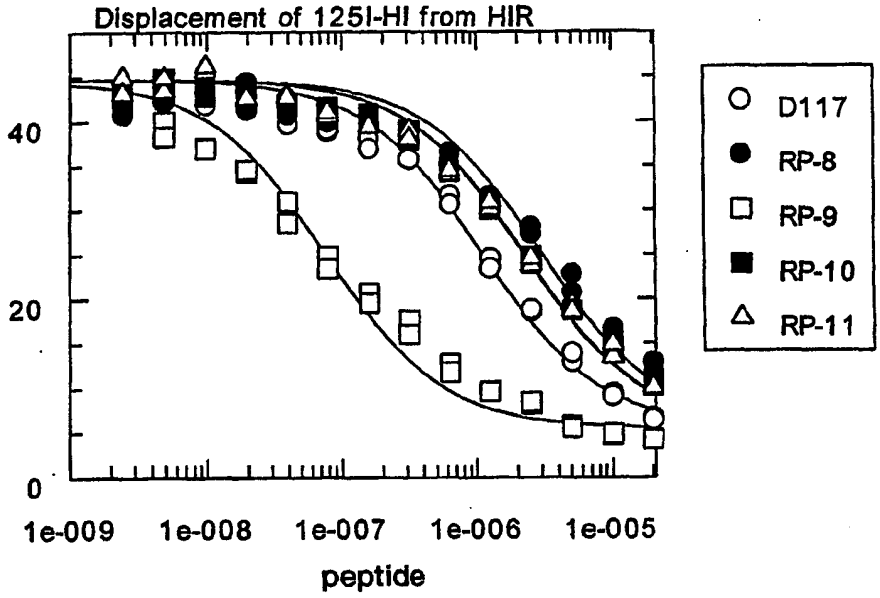


FIG. 70M

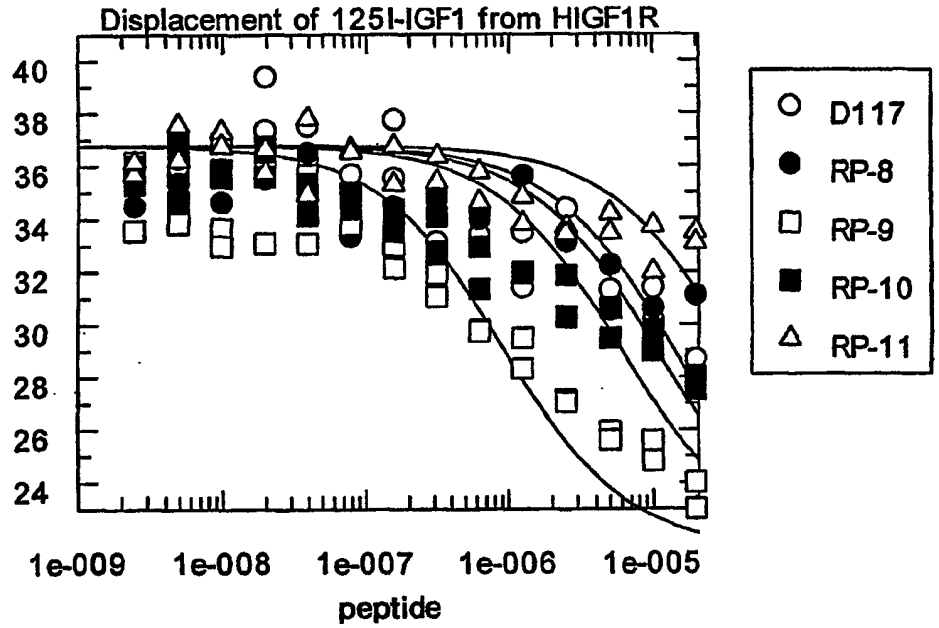


FIG. 70N

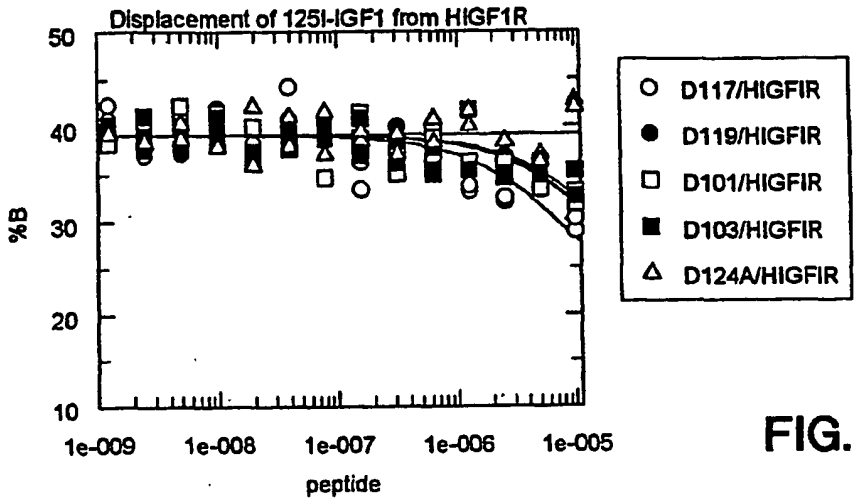


FIG. 70J

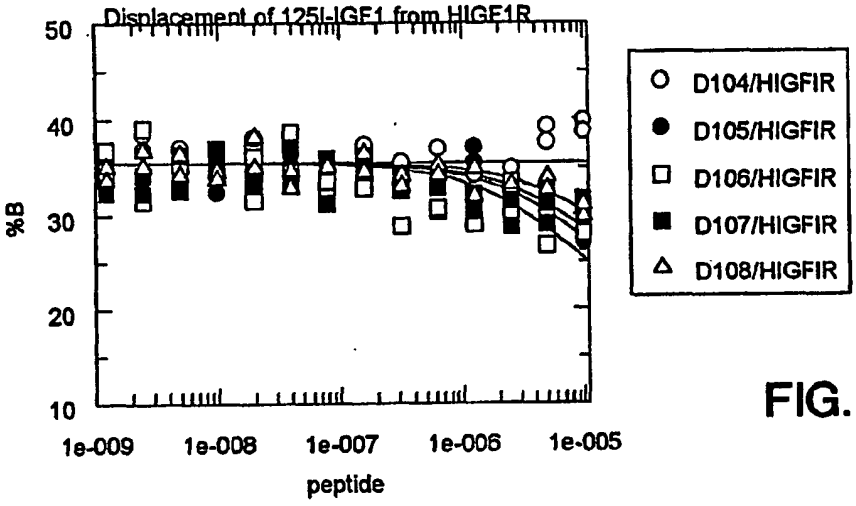


FIG. 70K

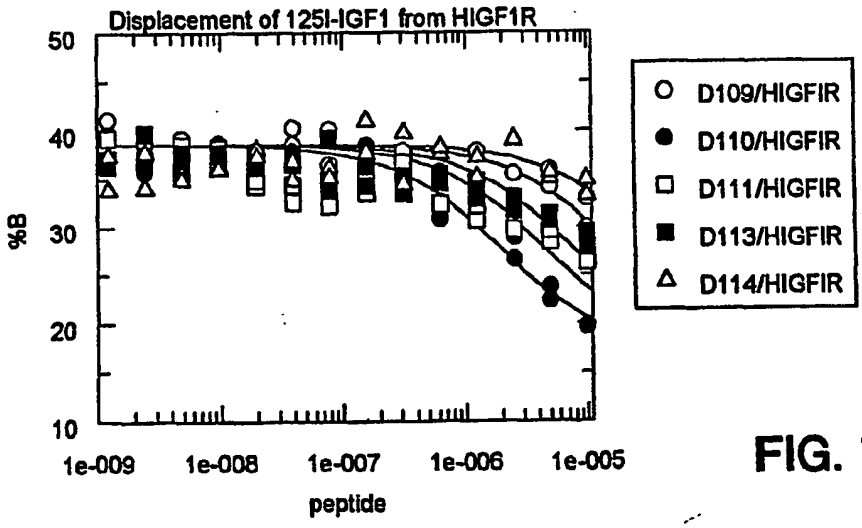


FIG. 70L

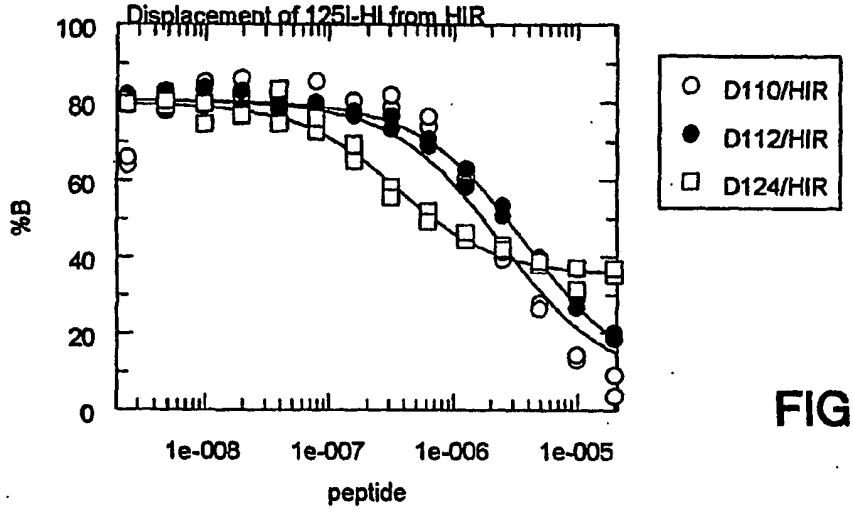


FIG. 70G

D990217A

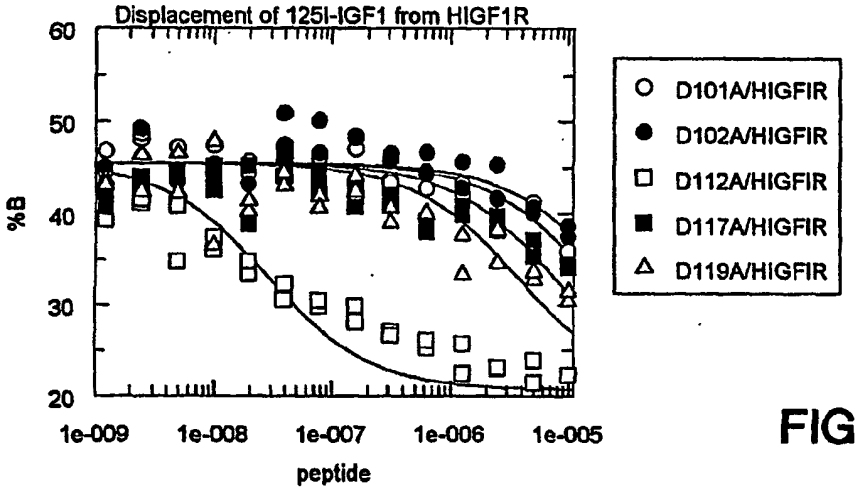


FIG. 70H

D990114A

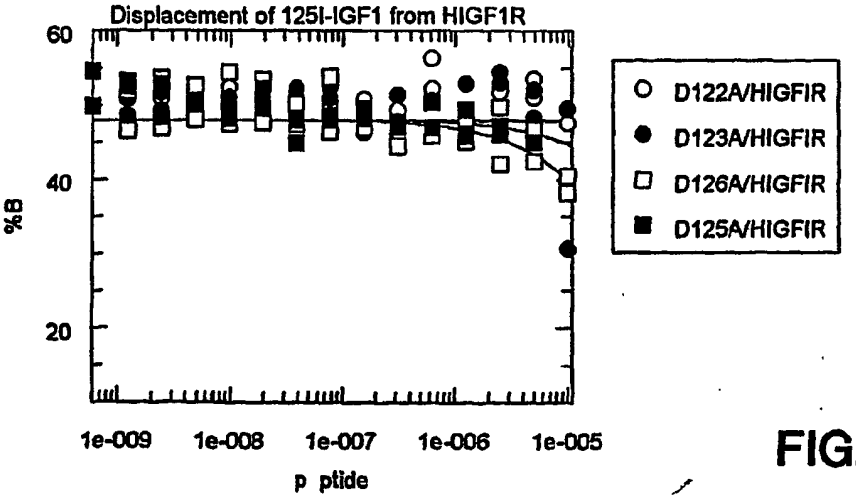


FIG. 70I

D990118A

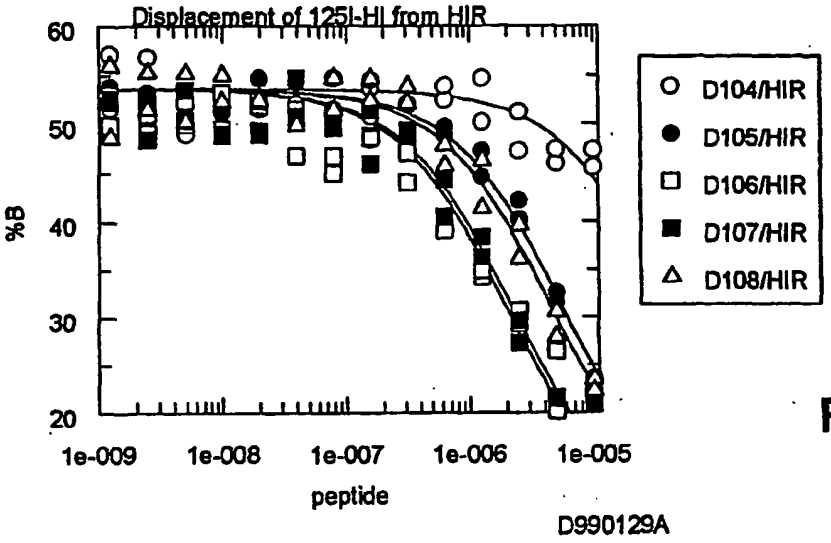


FIG. 70D

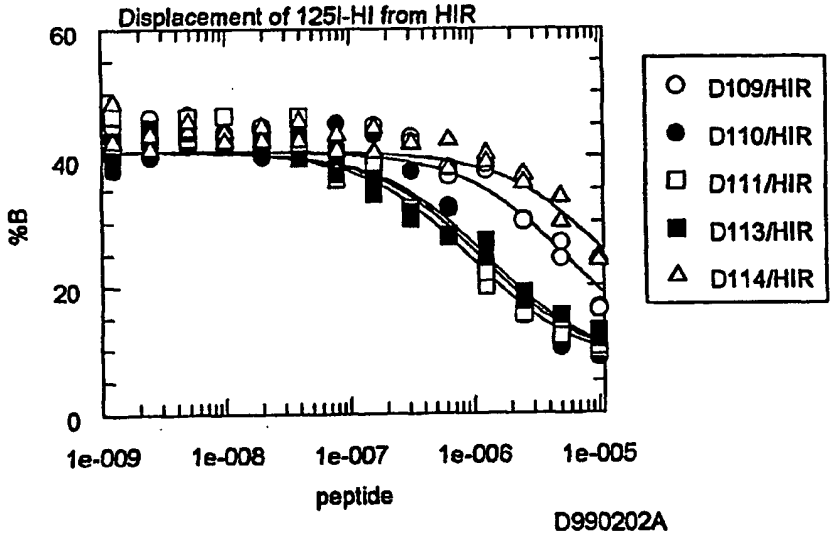


FIG. 70E

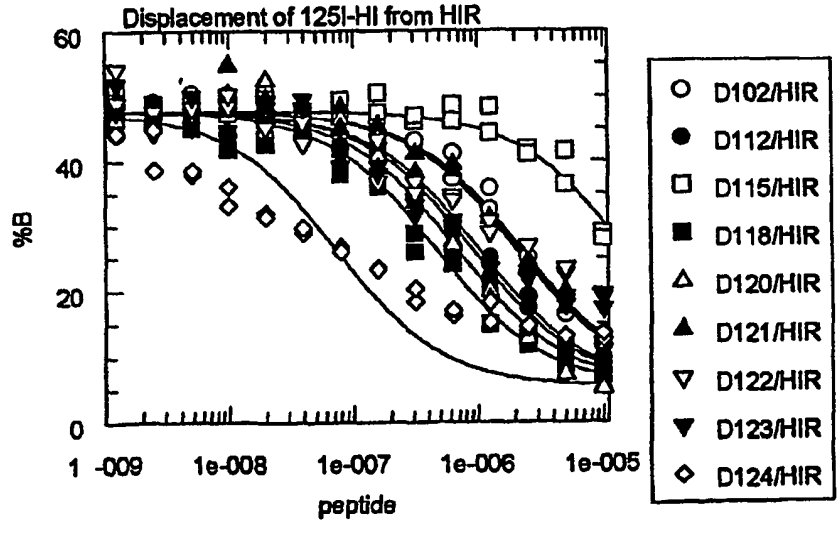


FIG. 70F

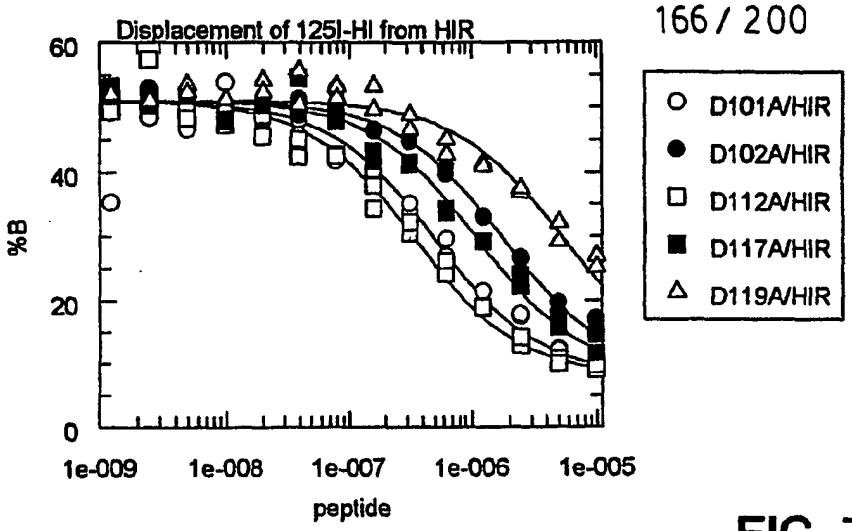


FIG. 70A

D990114A

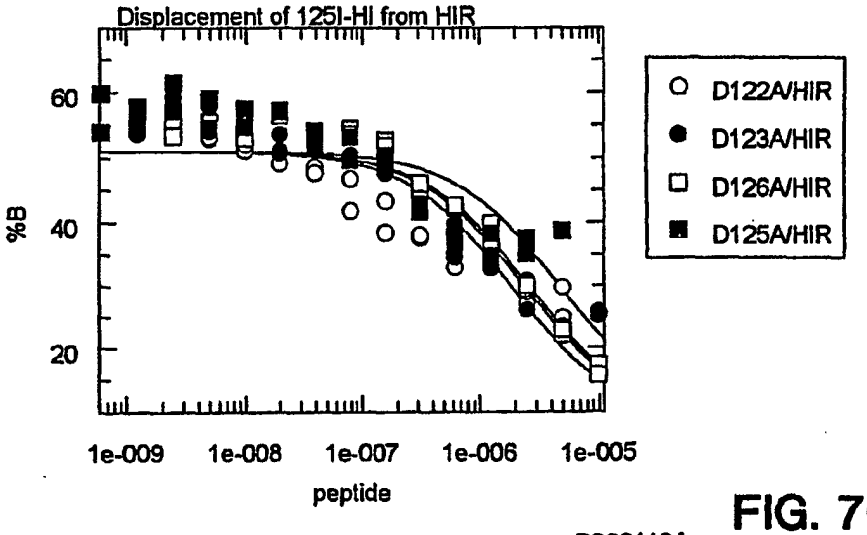


FIG. 70B

D990118A

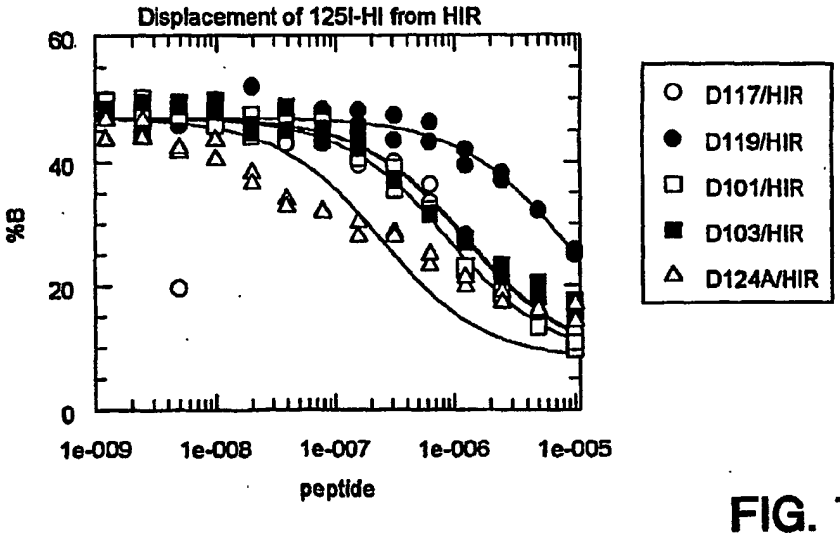


FIG. 70C

D990126A

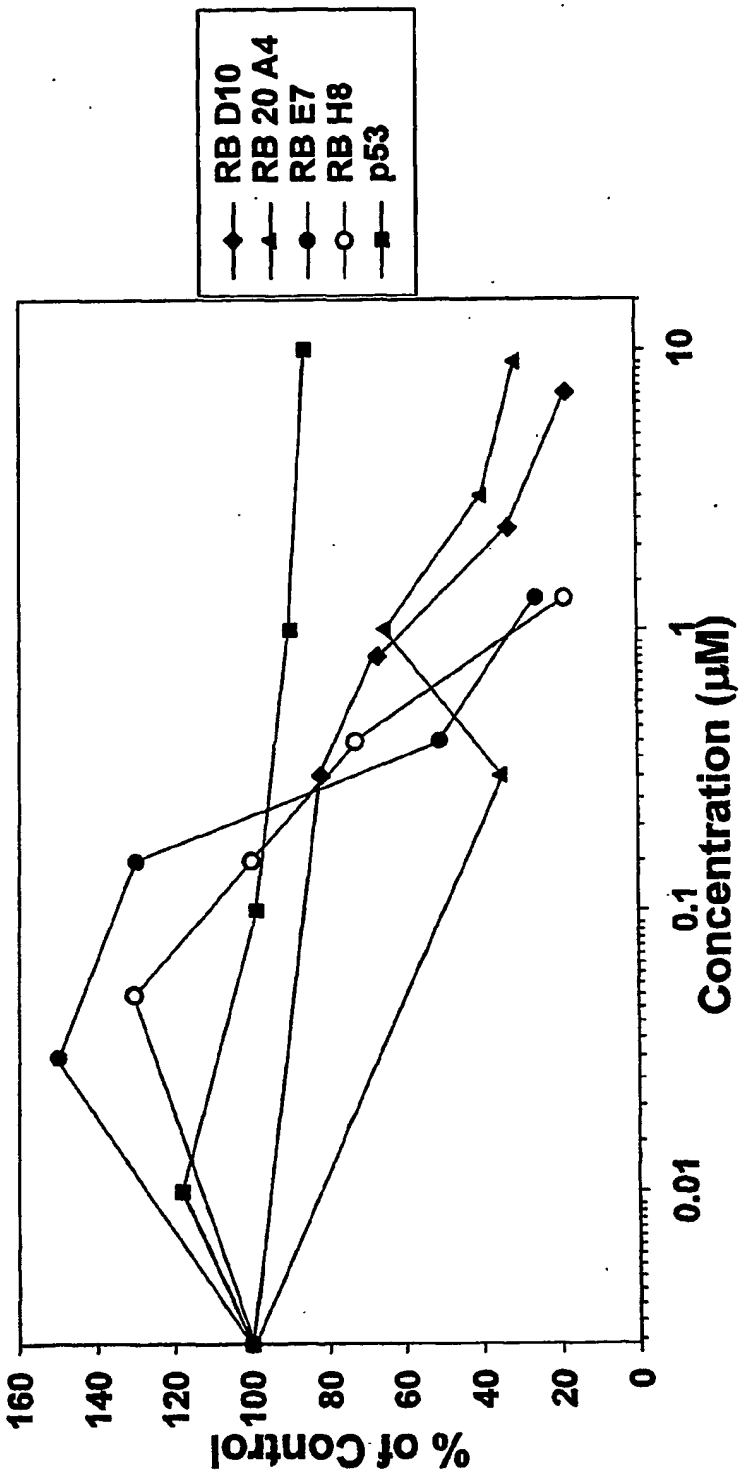

RB D10
RB 20 A4
RB E7
RB H8
p53
% of Control
160
140
120
100
80
60
40
20
0
0.01
0.1
1
10
Concentration (μM)

FIG. 69

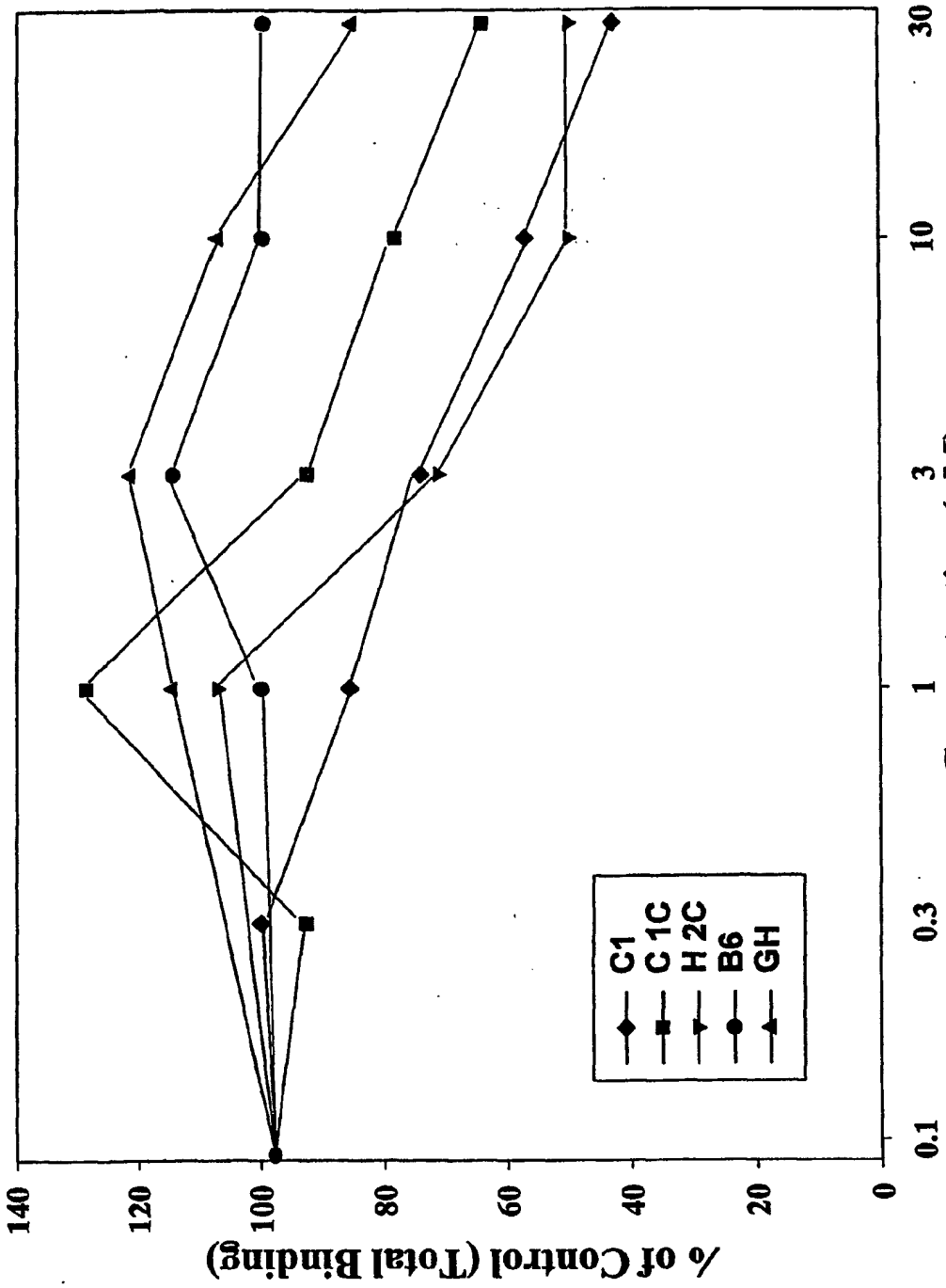

% of Control (Total Binding)
140
120
100
80
60
40
20
0
0.1
0.3
1
3
10
30
Concentration (µM)
C1
C1C
H2C
B6
GH

FIG. 68

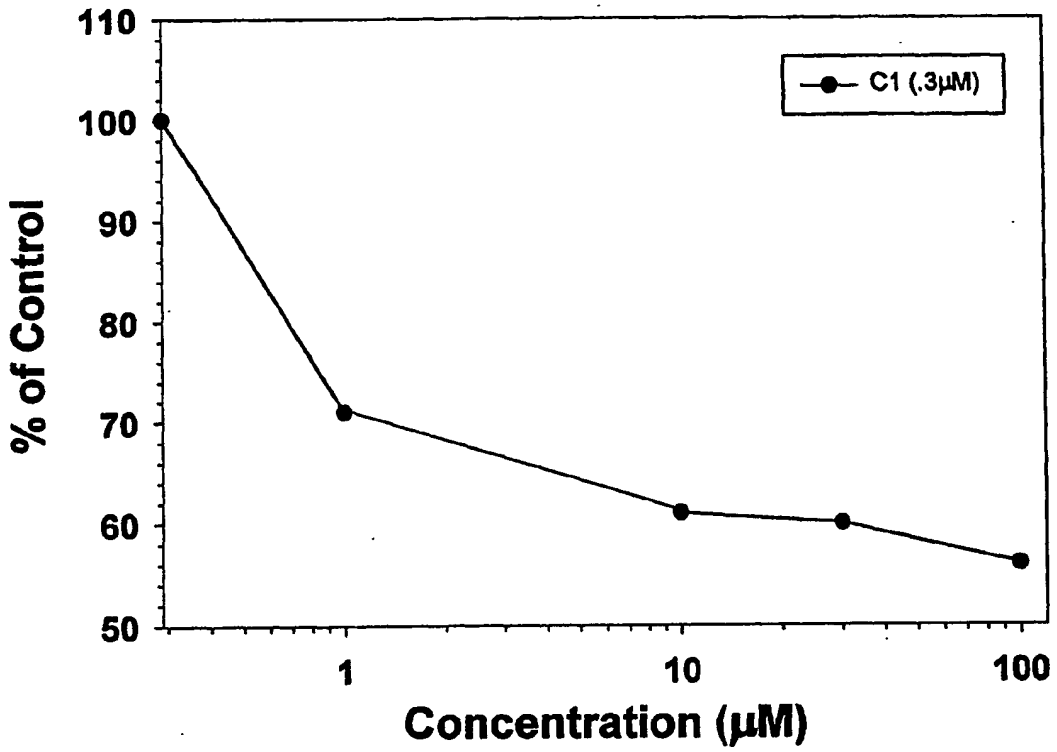

110
100
90
80
70
60
50
% of Control
C1 (.3μM)
1
10
100
Concentration (μM)

FIG. 67

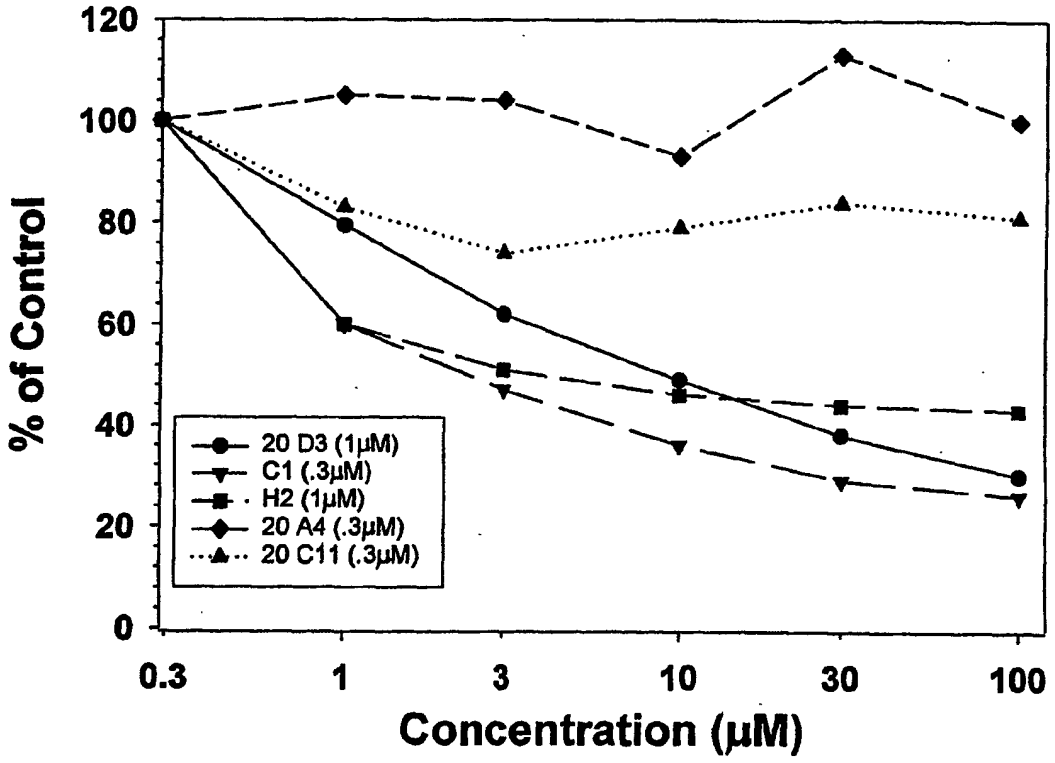

120
100
80
60
40
20
0
% of Control
0.3
1
3
10
30
100
Concentration (μM)
20 D3 (1μM)
C1 (.3μM)
H2 (1μM)
20 A4 (.3μM)
20 C11 (.3μM)

FIG. 66

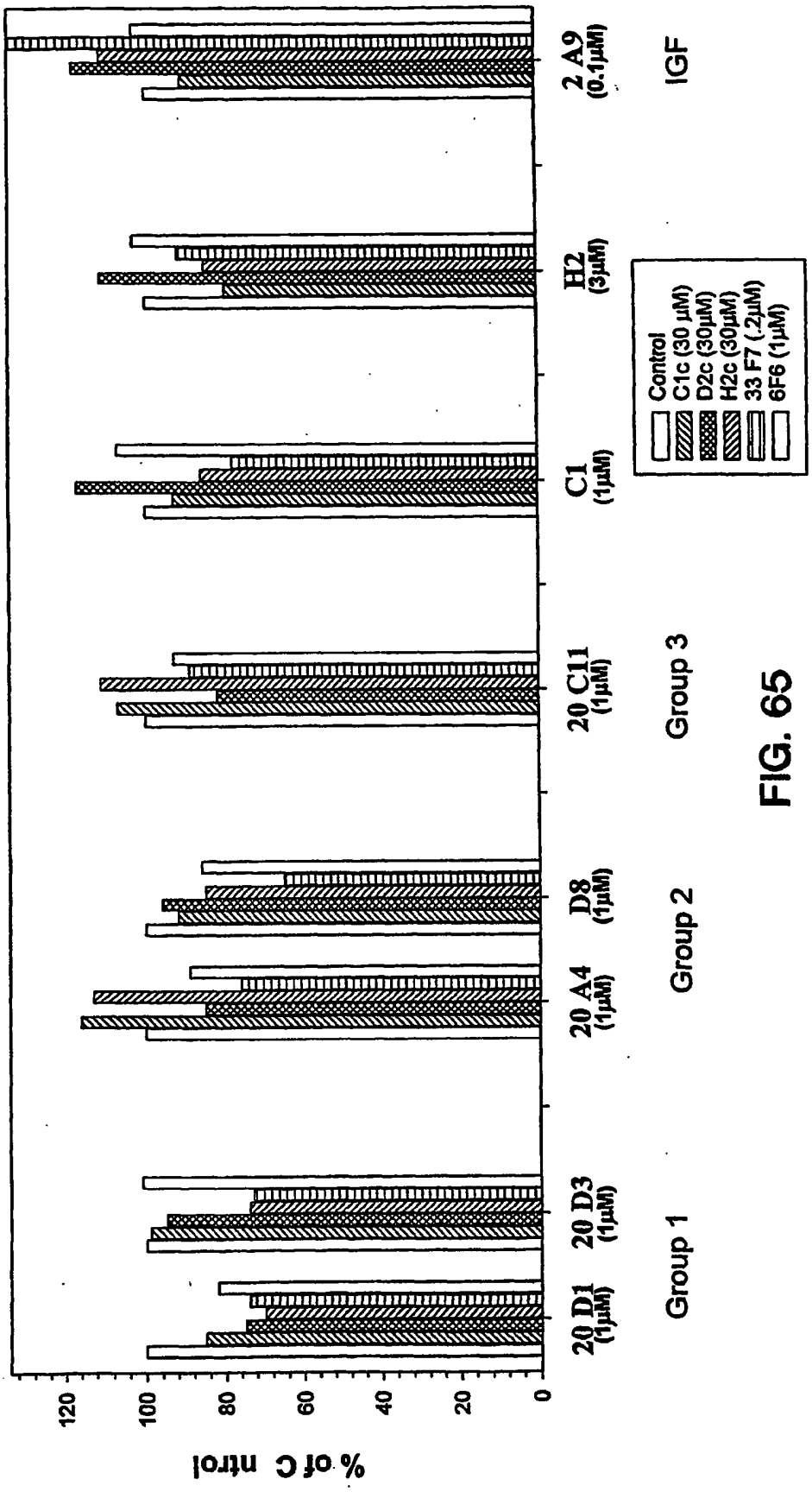

% of Control
120
100
80
60
40
20
0
20 D1 (1μM)
20 D3 (1μM)
20 A4 (1μM)
D8 (1μM)
20 C11 (1μM)
C1 (1μM)
H2 (3μM)
2 A9 (0.1μM)
Group 1
Group 2
Group 3
IGF
Control
C1c (30 μM)
D2c (30μM)
H2c (30μM)
33 F7 (.2μM)
6F6 (1μM)

FIG. 65

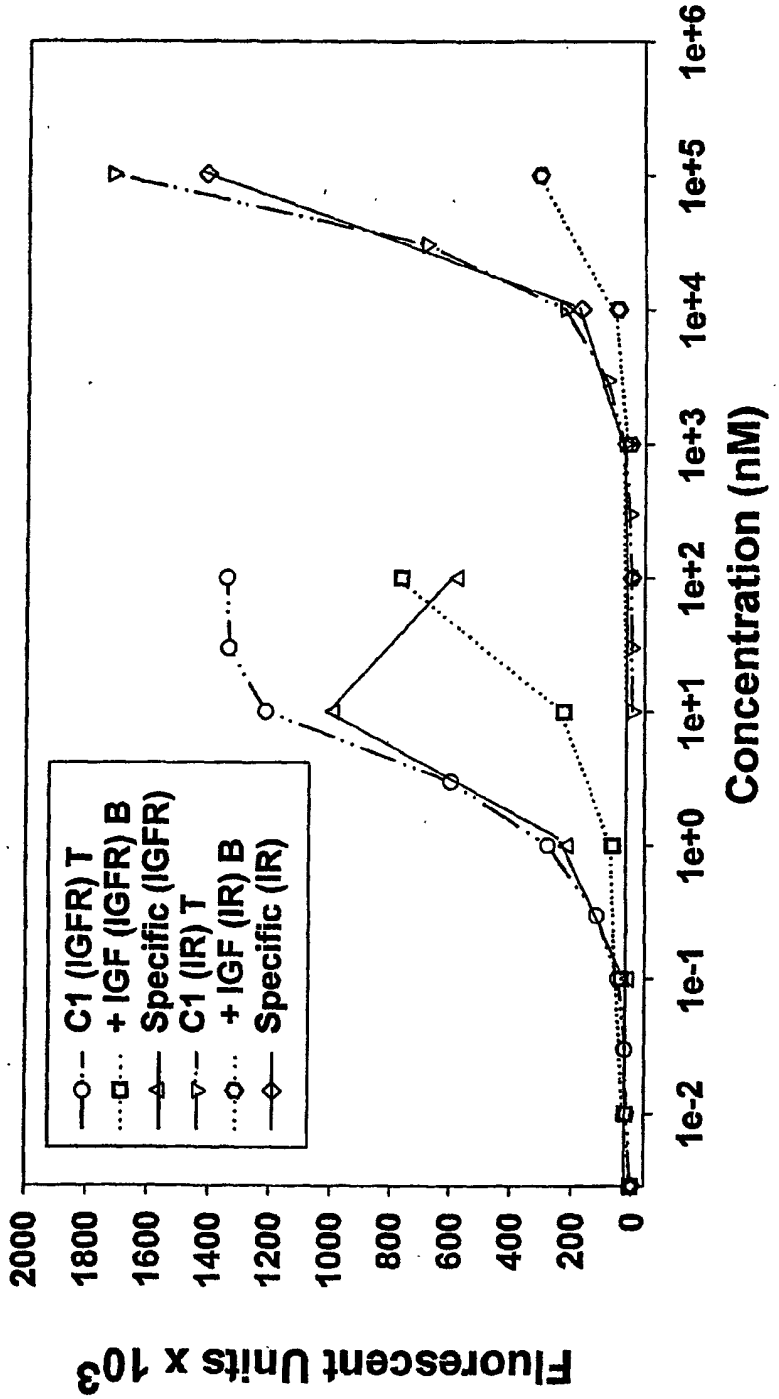

Fluorescent Units x 10³
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
Concentration (nM)
C1 (IGFR) T
+ IGF (IGFR) B
Specific (IGFR)
C1 (IR) T
+ IGF (IR) B
Specific (IR)

FIG. 64

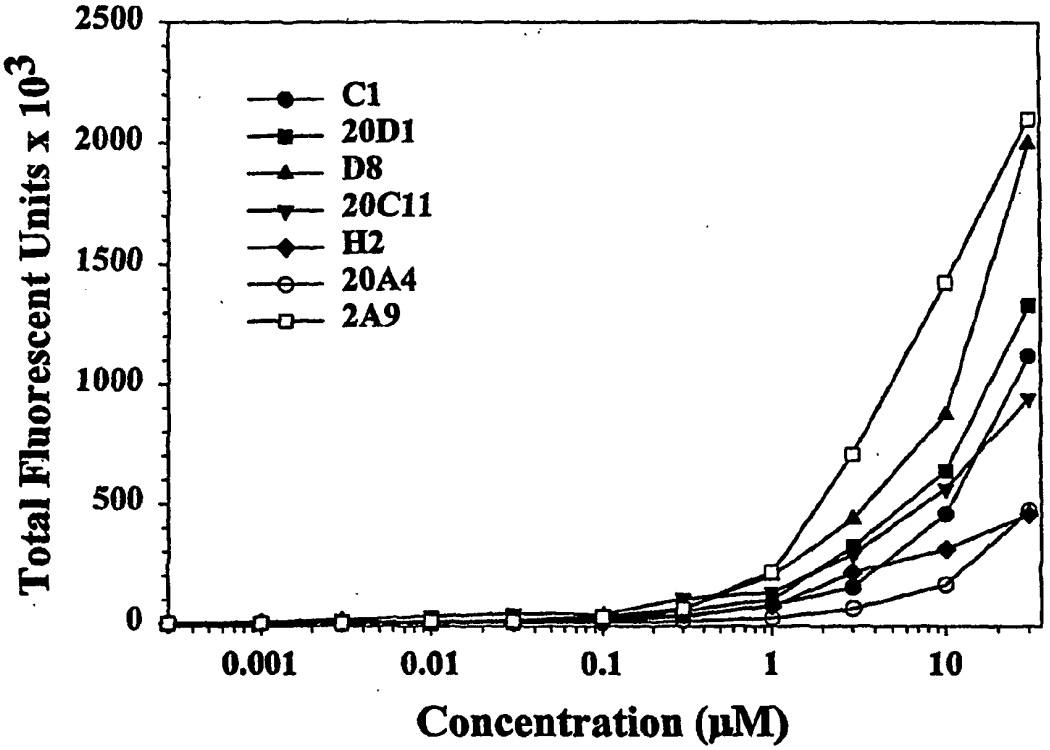

2500
2000
1500
1000
500
0
Total Fluorescent Units x 10^3
C1
20D1
D8
20C11
H2
20A4
2A9
0.001
0.01
0.1
1
10
Concentration (μM)

FIG. 63

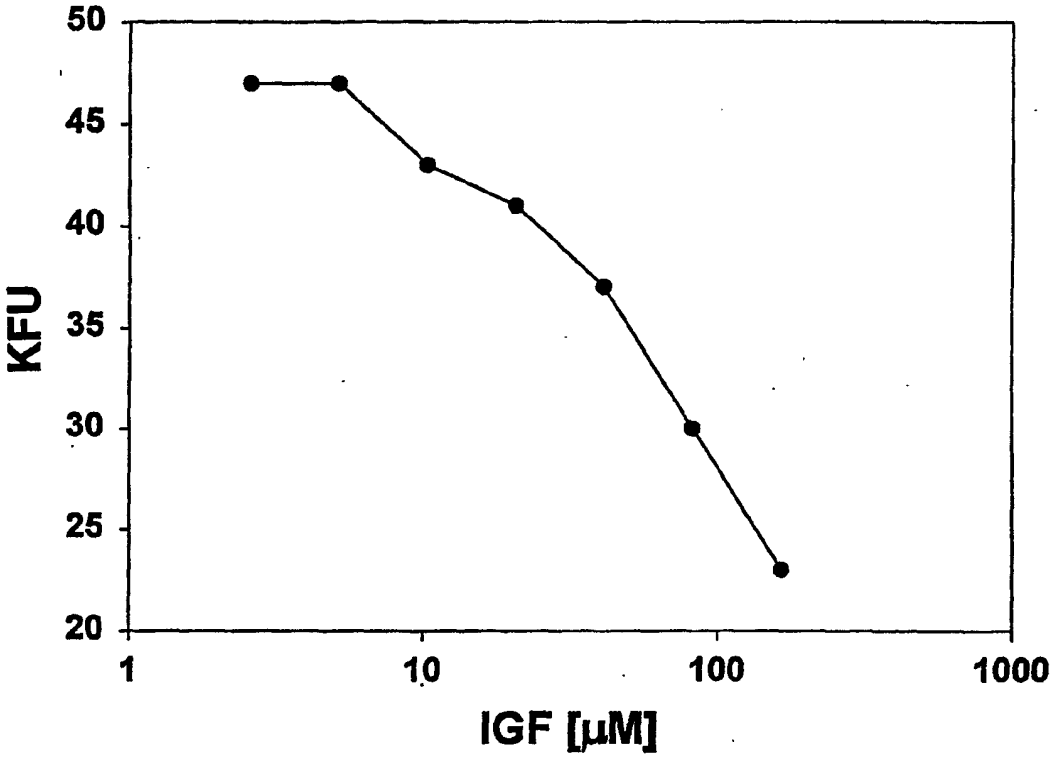


FIG. 62

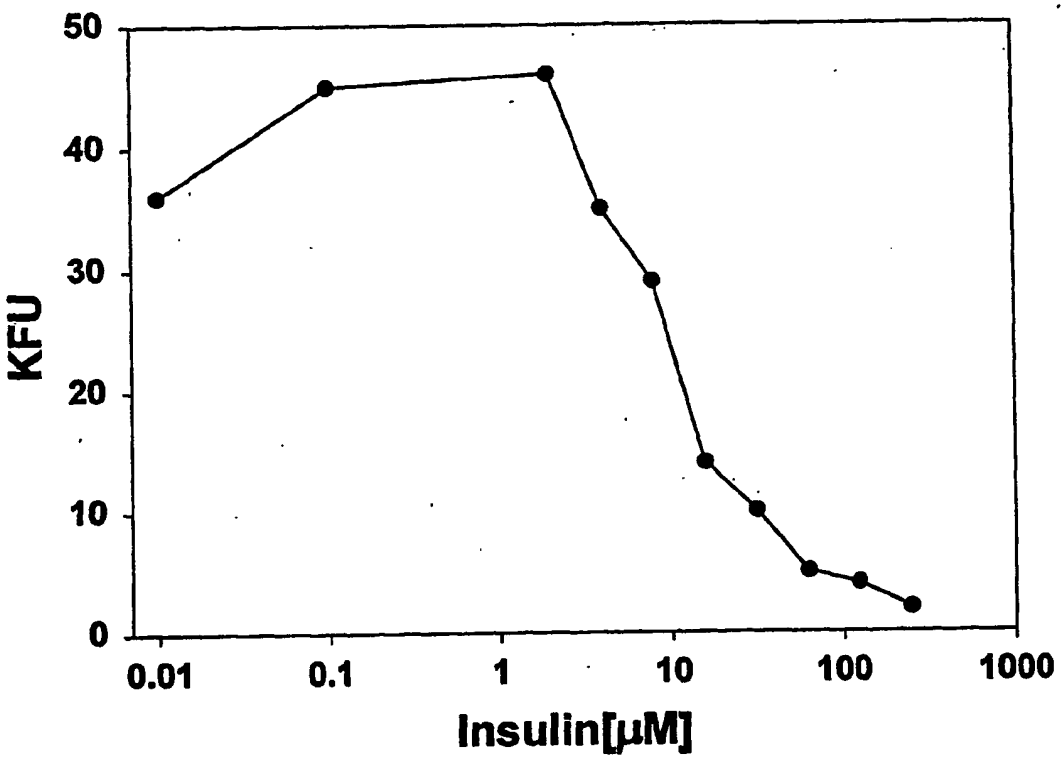


FIG. 61

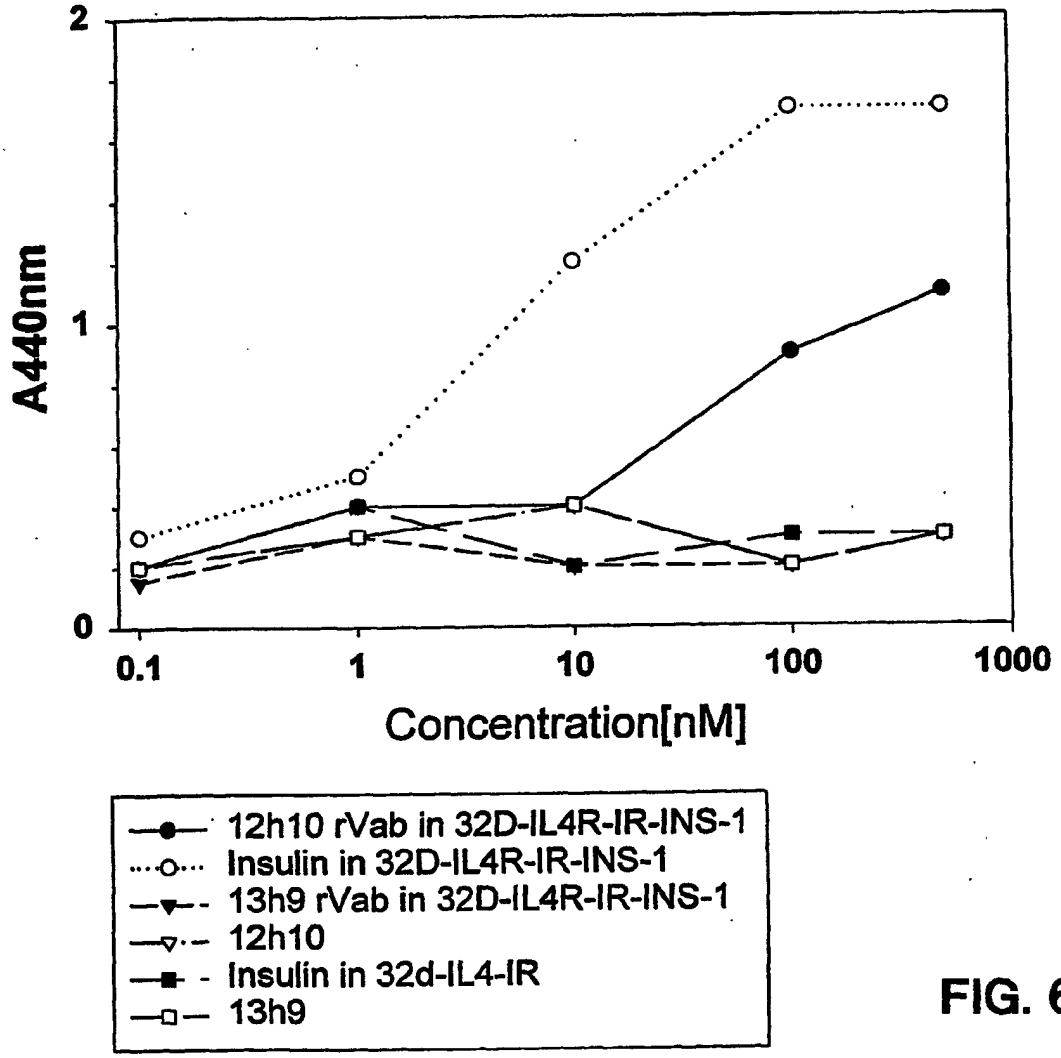

A440nm
2
1
0
0.1
1
10
100
1000
Concentration[nM]
12h10 rVab in 32D-IL4R-IR-INS-1
Insulin in 32D-IL4R-IR-INS-1
13h9 rVab in 32D-IL4R-IR-INS-1
12h10
Insulin in 32d-IL4-IR
13h9

FIG. 60

CLONES	VHCDR3	%Inhibition Activity
118:	PFFV FYRGQDT	54%
?		
InsulB:	FVNQHLCGSHLVEALY LVCGERGFFYTPKT	
12H10:	C VVYNYA G RG T	42%
Ang?		
13-e-4:	VQAWDGL G RES	52%
?		
13h9:	GGL G RRDWL	30%
?		
24:	GGRR H RLG	
InsulinA	GIVEQCCTSICSLYQ LENYCN	
11a8:	ENYGNSE	32%
?		
	GDQELQNY	None
N/A		

FIG. 59

```
GCCCATCCGG CCATGGCCGA GGTGCAGCTG TTGGAGTCTG GGGGAGGCTT GGTAAAGCCT   60
                    E  V  Q  L  L  E  S  G  G  G  L  V  K  P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG  120
G  G  S  L  R  L  S  C  A  A  S  G  F  T  F  S  N  A  W  M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA  180
S  W  V  R  Q  A  P  G  K  G  L  E  W  V  G  R  I  K  S  K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA  240
T  D  G  G  T  T  D  Y  A  A  P  V  K  G  R  F  T  I  S  R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC  300
D  D  S  K  N  T  L  Y  L  Q  M  N  S  L  K  T  E  D  T  A

GTGTATTACT GTACCACATA CGATTAGTGG GGGGTCTTGG TGGTCTGGGG TCAAGGAACT  360
V  Y  Y  C  T  T  Y  D  .  W  G  V  L  V  V  W  G  Q  G  T

CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA  420
L  V  T  V  S  S  G  G  G  G  S  G  G  G  G  S  G  G  G

TCCGACATCC AGTTGACCCA GTCTCCATCC TTCCTGTCTG CATCTGTAGG AGACAGAGTC  480
S  D  I  Q  L  T  Q  S  P  S  F  L  S  A  S  V  G  D  R  V

ACCATCACTT GCCGGGCCAG TCAGGGCATT AGCAGTTATT TAGCCTGGTA TCAGCAAAAA  540
T  I  T  C  R  A  S  Q  G  I  S  S  Y  L  A  W  Y  Q  Q  K

CCAGGGAAAG CCCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAAAG TGGGGTCCCA  600
P  G  K  A  P  K  L  L  I  Y  A  A  S  T  L  Q  S  G  V  P

TCAAGGTTCA GCGGCAGTGG ATCTGGGACA GATTTCACTC TCACCATCAG CAGCCTGCAG  660
S  R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q

CCTGAAGATT TTGCAACTTA TTACTGTCAA CAGCTTAATA GTTACCCTTT CACTTTCGGC  720
P  E  D  F  A  T  Y  Y  C  Q  Q  L  N  S  Y  P  F  T  F  G

CCTGGGACCA AAGTGGATAT CAAAGCGGCC GCAGGTGC                          758
P  G  T  K  V  D  I  K
```

FIG. 58

```
GCCCAGCCGG CCATGGCCGA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT   60
                    E   V   Q   L   V   E   S   G   G   G   L   V   K   P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG  120
G   G   S   L   R   L   S   C   A   A   S   G   F   T   F   S   N   A   W   M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA  180
S   W   V   R   Q   A   P   G   K   G   L   E   W   V   G   R   I   K   S   K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA  240
T   D   G   G   T   T   D   Y   A   A   P   V   K   G   R   F   T   I   S   R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC  300
D   D   S   K   N   T   L   Y   L   Q   M   N   S   L   K   T   E   D   T   A

GTGTATTACT GTACCACATA CGGCGACGTT TACGACCGCG ATTACGATGG GCGCTGGGGT  360
V   Y   Y   C   T   T   Y   G   D   V   Y   D   R   D   Y   D   G   R   W   G

CAAGGAACTC TGGTCACCGT CTCCTCAGGT GGAGGCGGTT CAGGCGGAGG TGGCTCTGGC  420
Q   G   T   L   V   T   V   S   S   G   G   G   G   S   G   G   G   G   S   G

GGTGGCGGAT CCGACATCCA GATGACCCAG TCTCCATCCT CCCTGTCTGC ATCTGTAGGA  480
G   G   G   S   D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G

GACAGAGTCA CCATCACTTG CCGGGCGAGT CAGGGCATTA GCAATTATTT AGCCTGGTAT  540
D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y   L   A   W   Y

CAGCAGAAAC CAGGGAAAGT TCCTAAGCTC CTGATCTATG CTGCATCCAC TTTGCAATCA  600
Q   Q   K   P   G   K   V   P   K   L   L   I   Y   A   A   S   T   L   Q   S

GGGGTCCCAT CTCGGTTCAG TGGCAGTGGA TCTGGGACAG ATTTCACTCT CACCATCAGC  660
G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S

AGCCTGCAGC CTGAAGATGT TGCAACTTAT TACTGTCAAA AGTATAACAG TGCCCCTTTC  720
S   L   Q   P   E   D   V   A   T   Y   Y   C   Q   K   Y   N   S   A   P   F

ACTTTCGGCC CTGGGACCAA AGTGGATATC AAAACGGCCG C                      761
T   F   G   P   G   T   K   V   D   I   K
```

FIG. 57

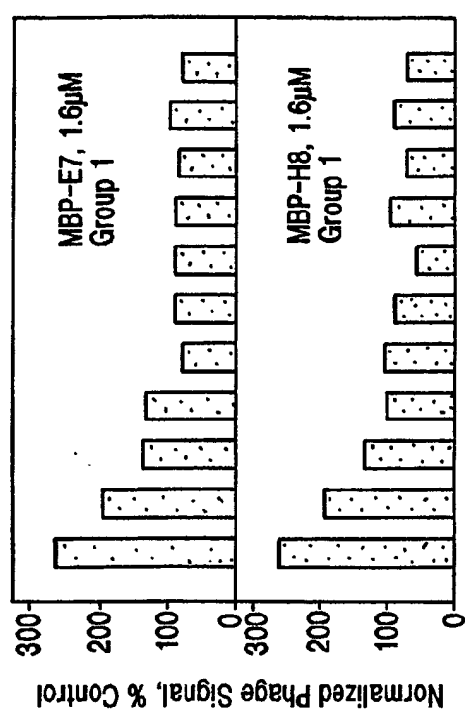


FIG. 56B

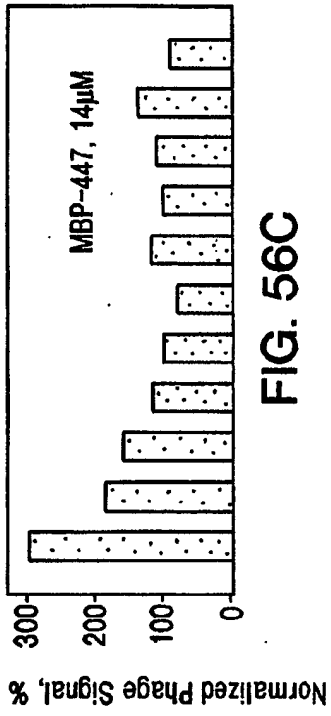


FIG. 56C

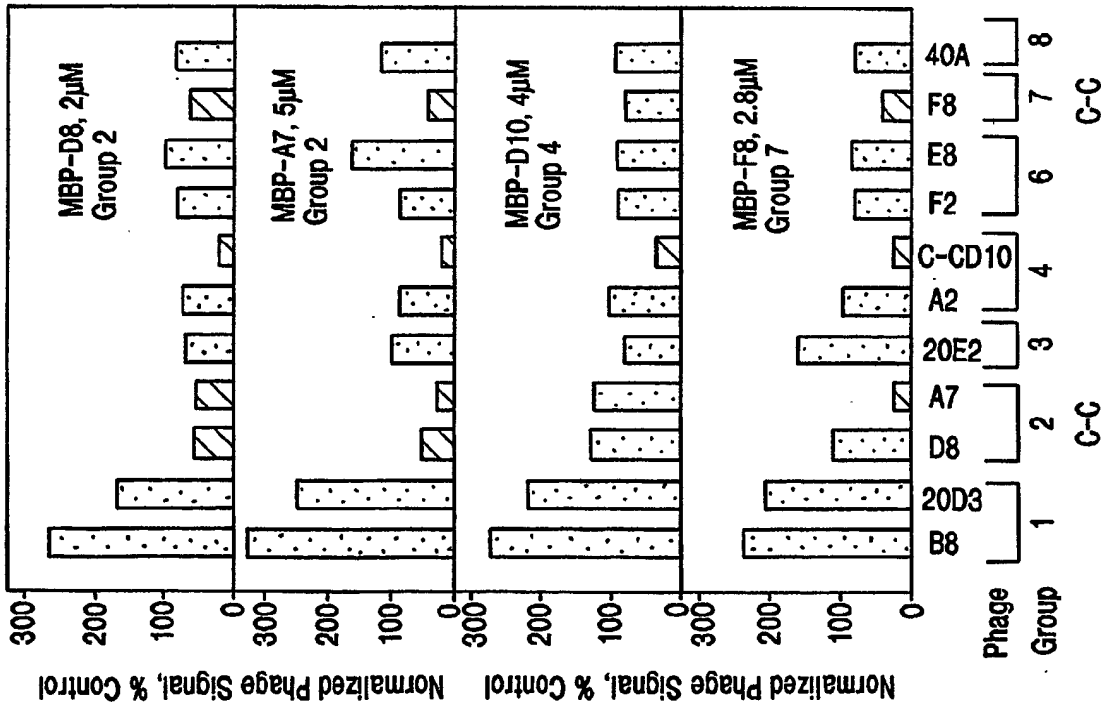


FIG. 56A

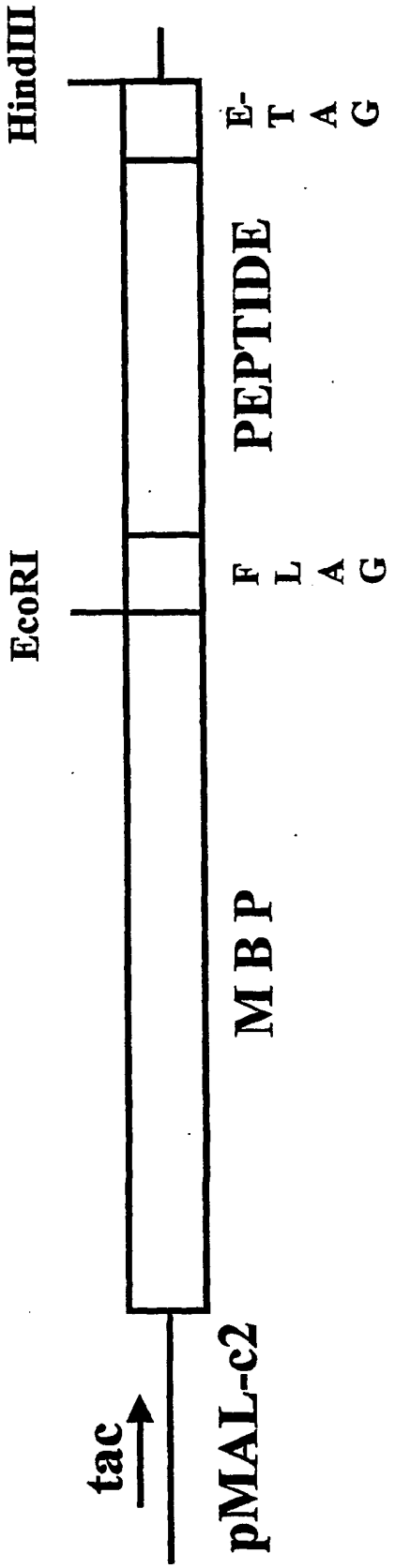


FIG. 55

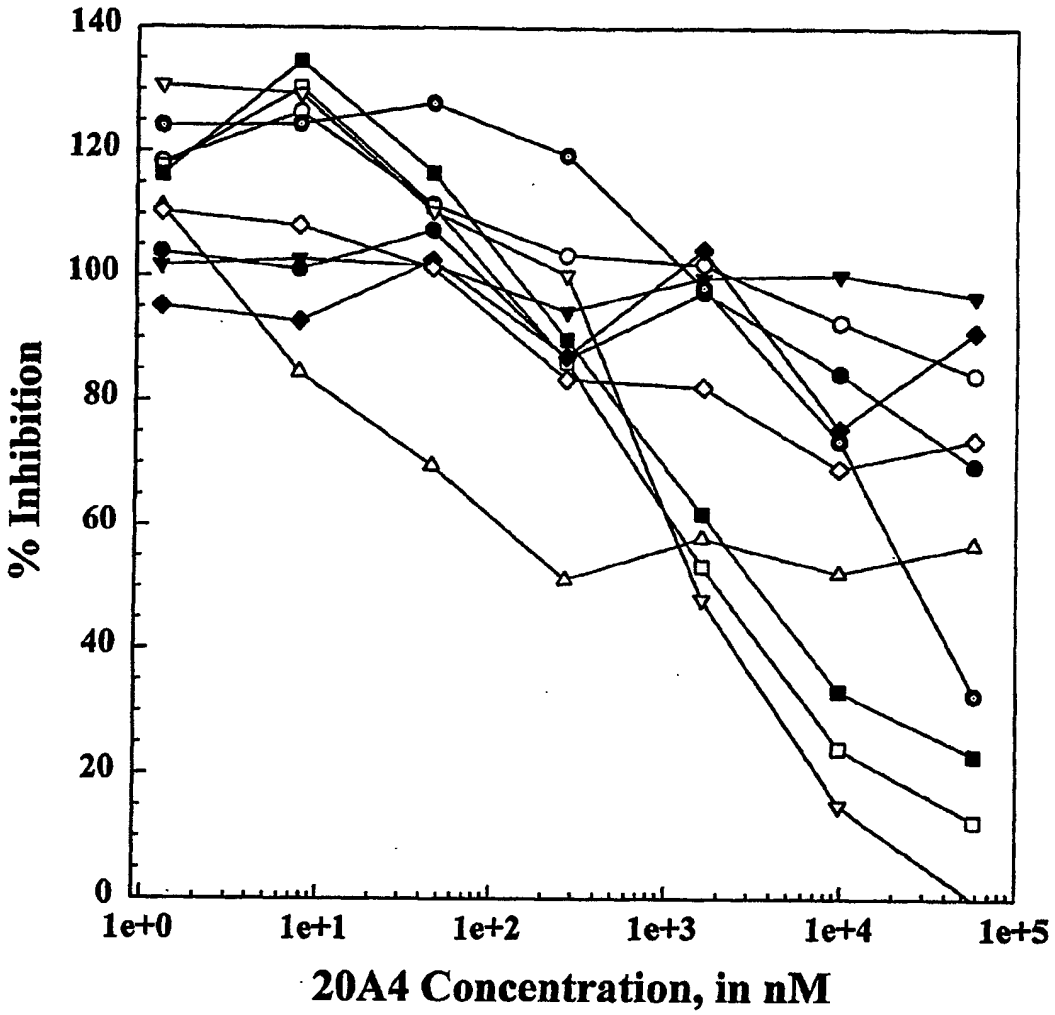

140
120
100
80
60
40
20
0
% Inhibition
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
20A4 Concentration, in nM

FIG. 54

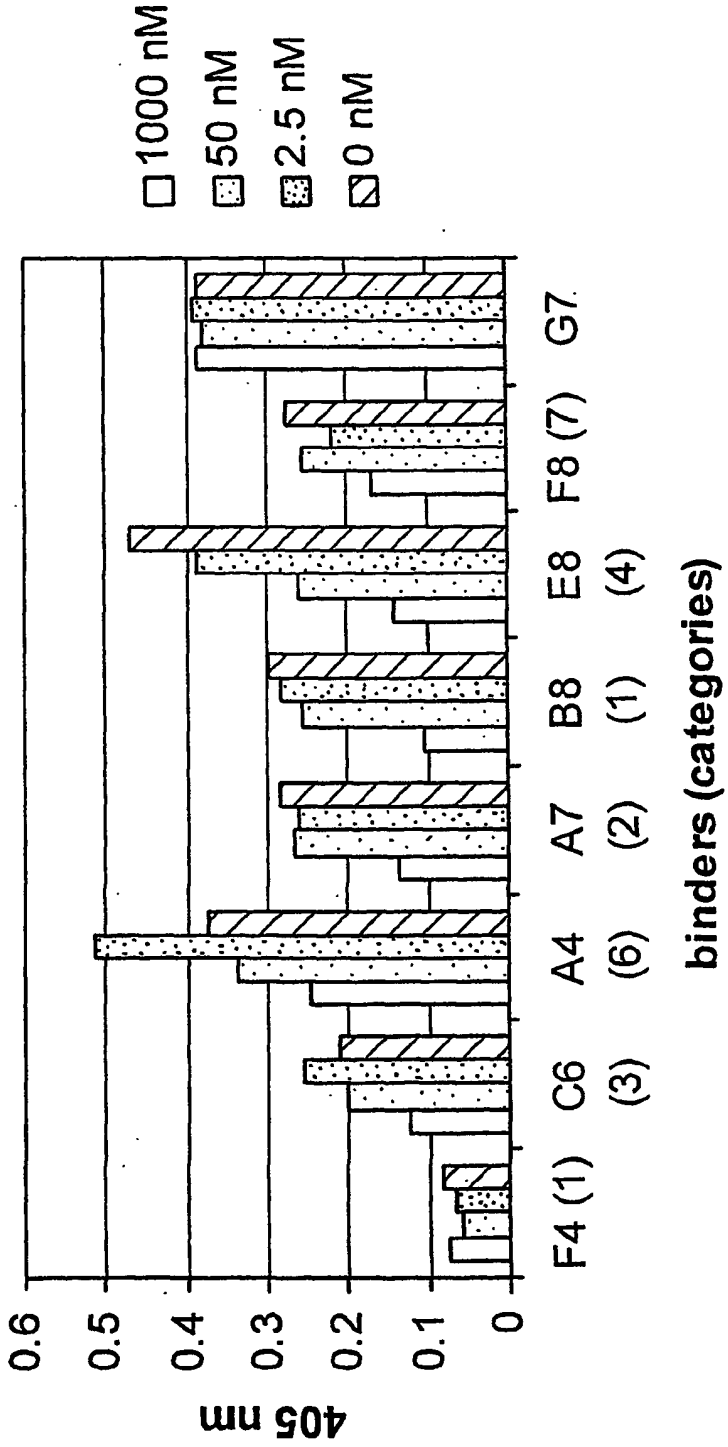

1000 nM
50 nM
2.5 nM
0 nM
405 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
F4 (1)
C6 (3)
A4 (6)
A7 (2)
B8 (1)
E8 (4)
F8 (7)
G7
binders (categories)

FIG. 53

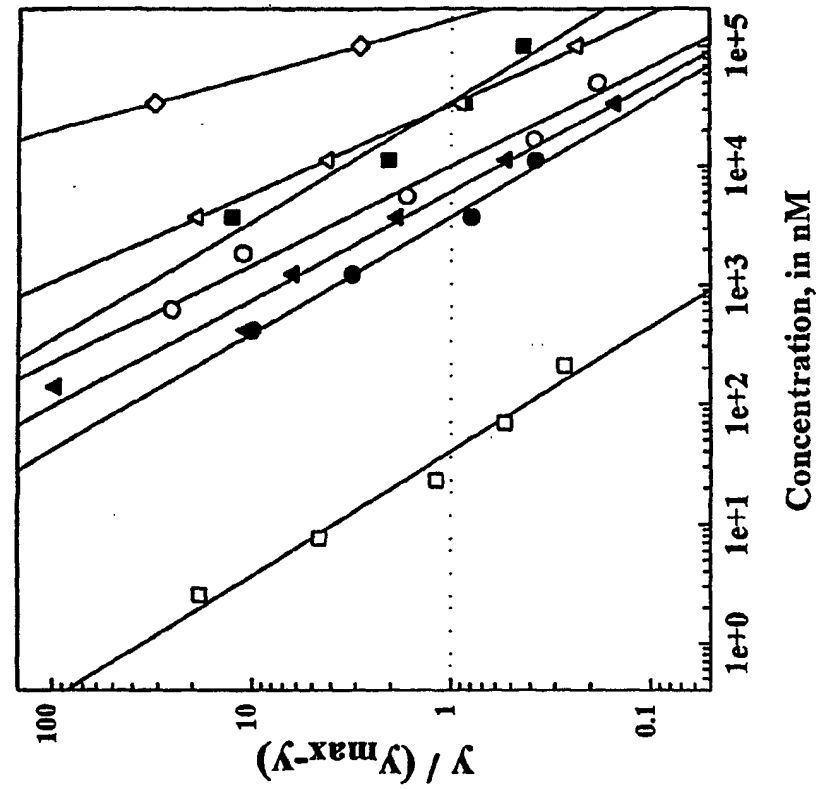


FIG. 52D

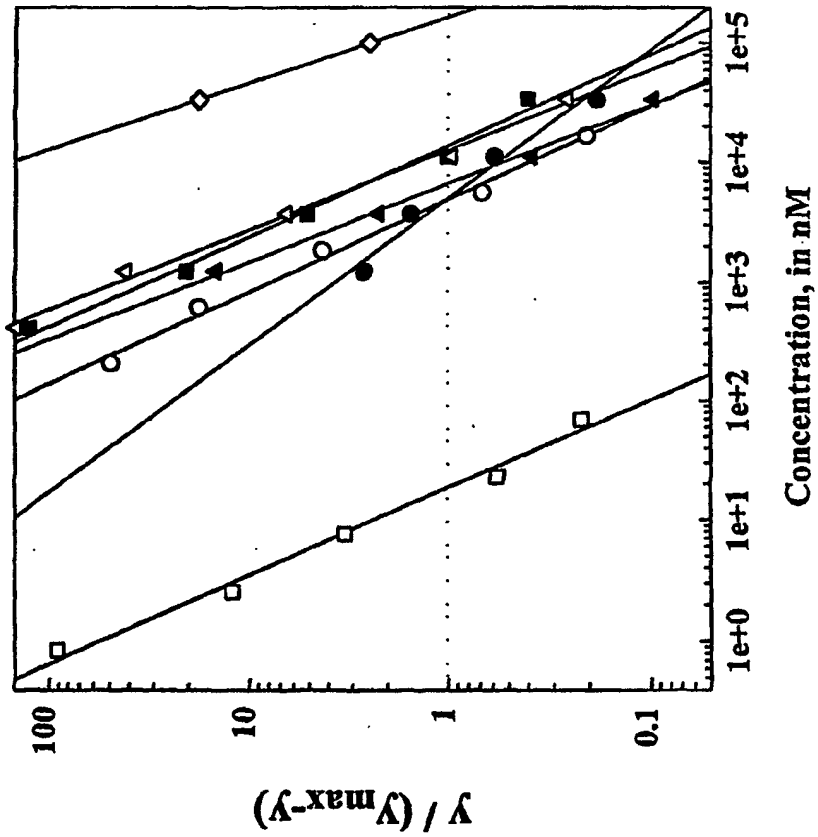


FIG. 52C

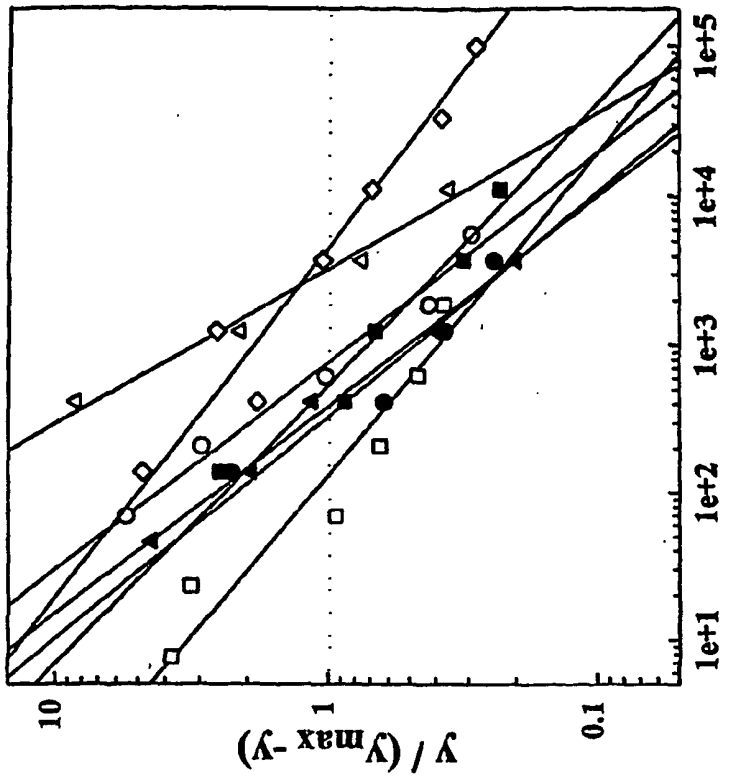


FIG. 52B

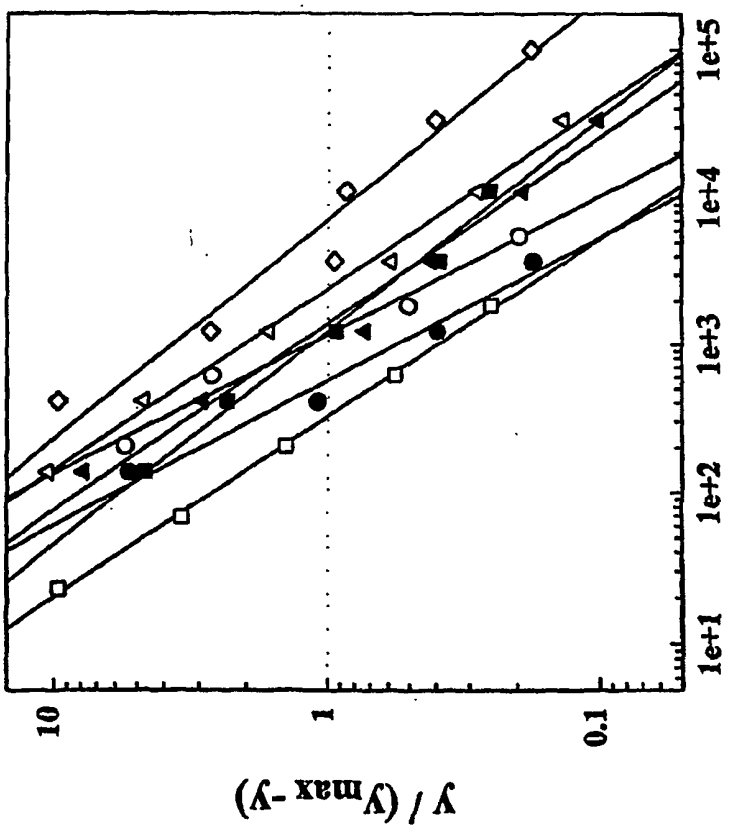


FIG. 52A

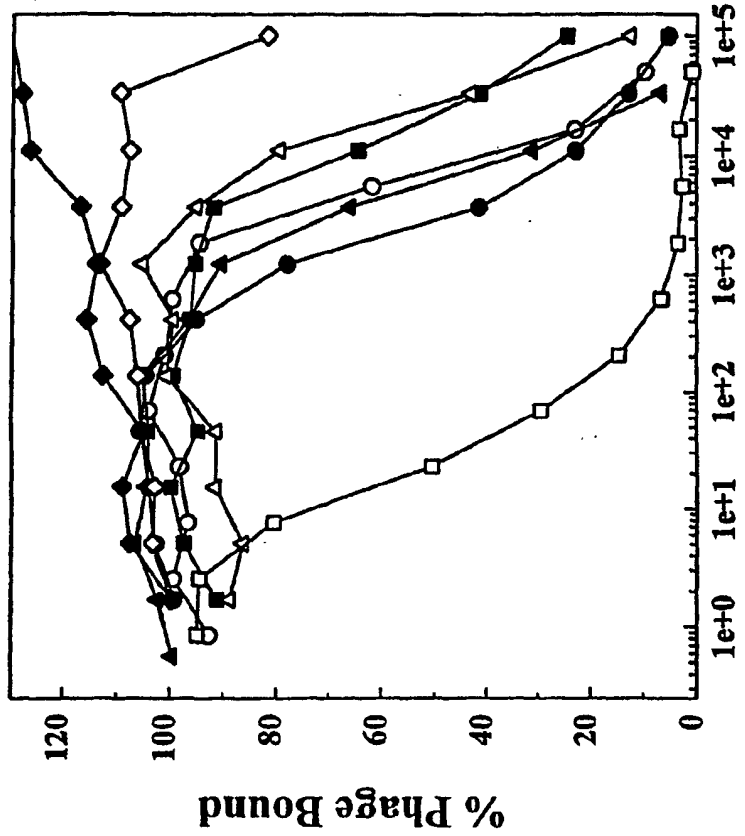


FIG. 51D

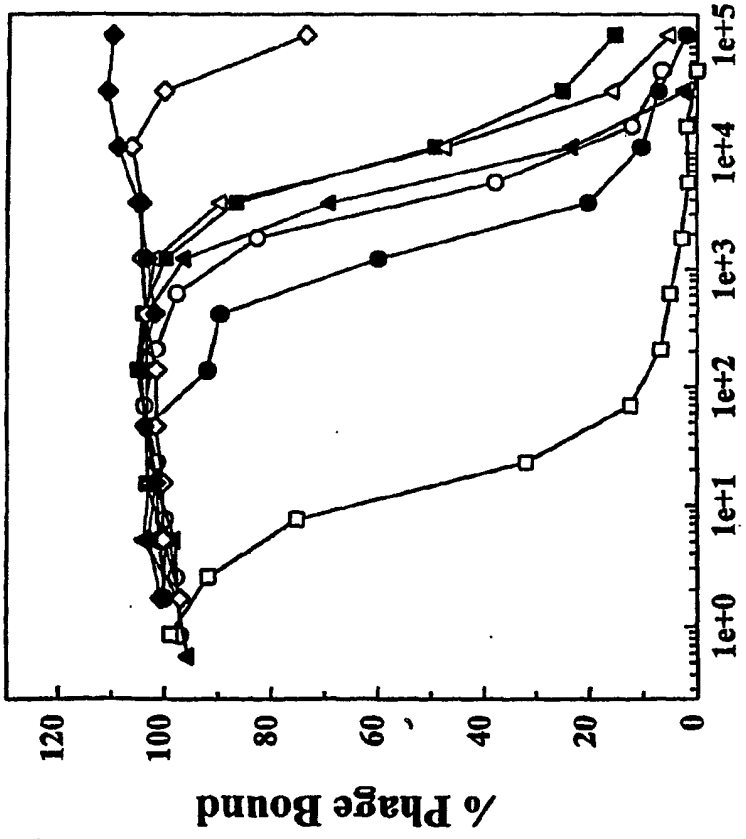


FIG. 51C

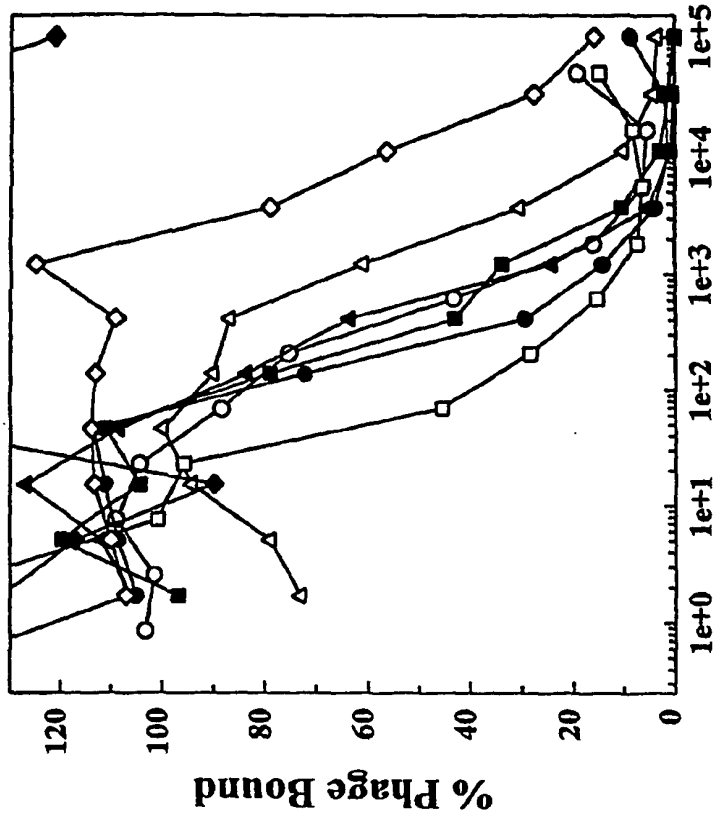


FIG. 51B

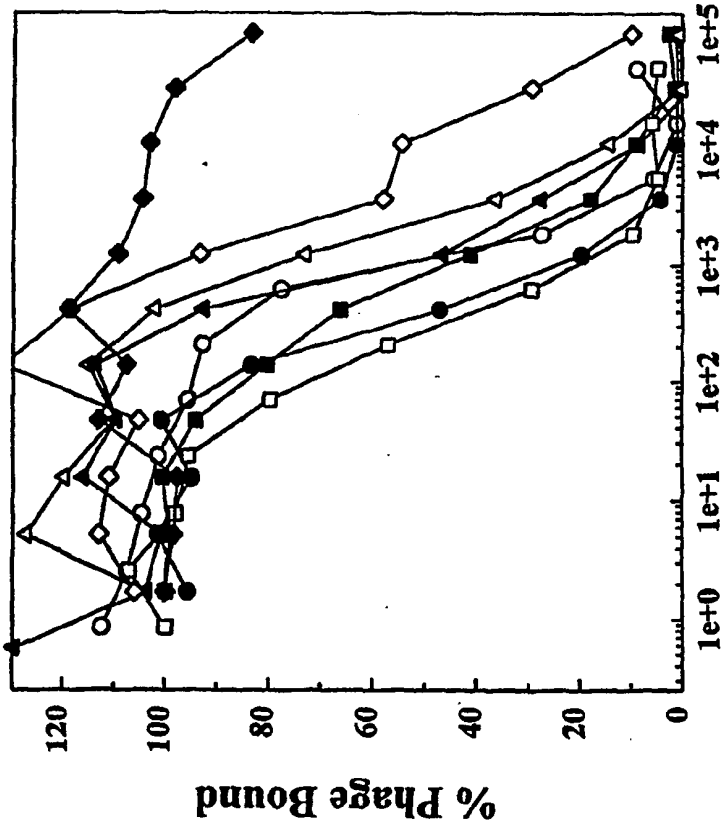


FIG. 51A

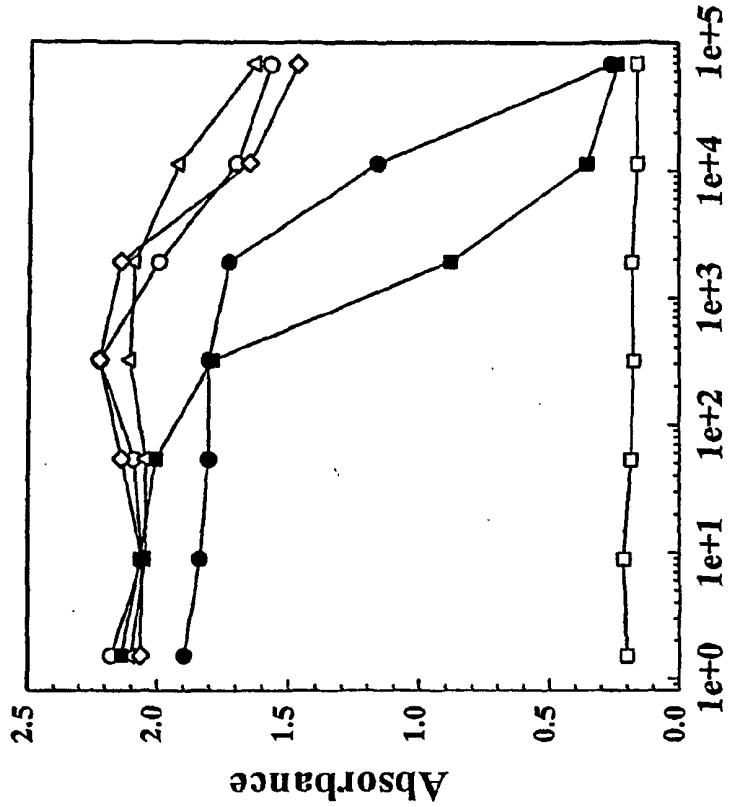


FIG. 50D

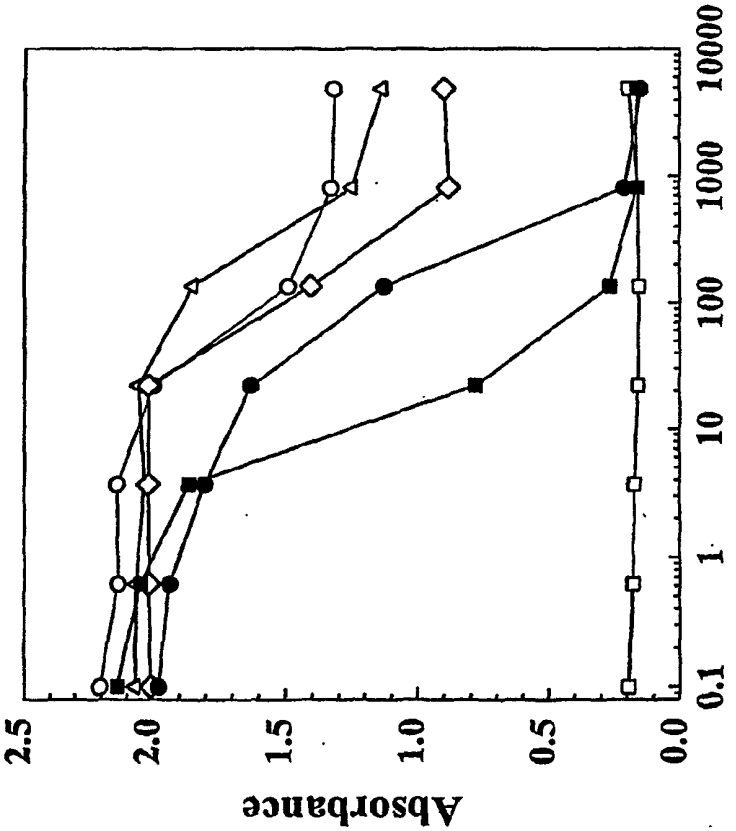


FIG. 50C

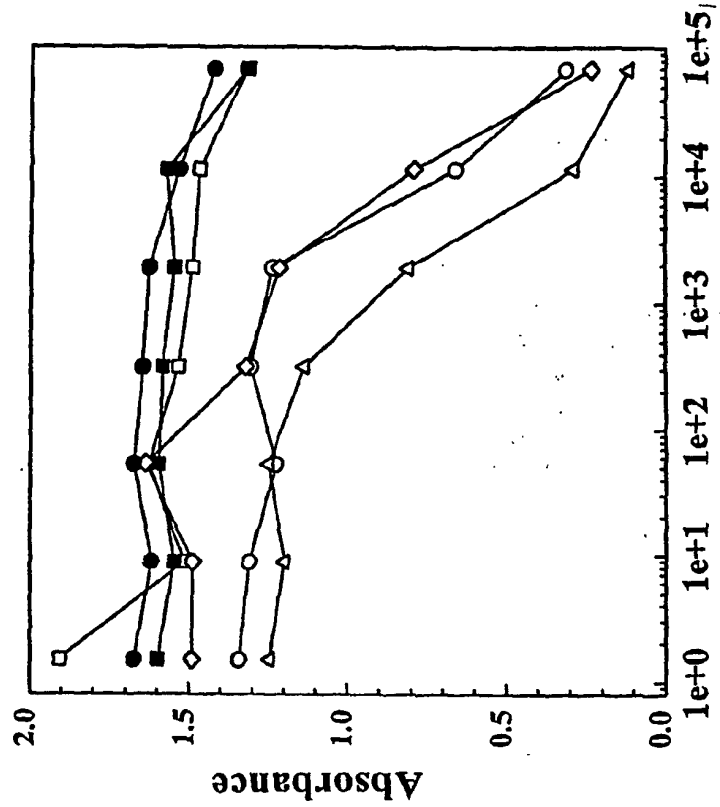


FIG. 50B

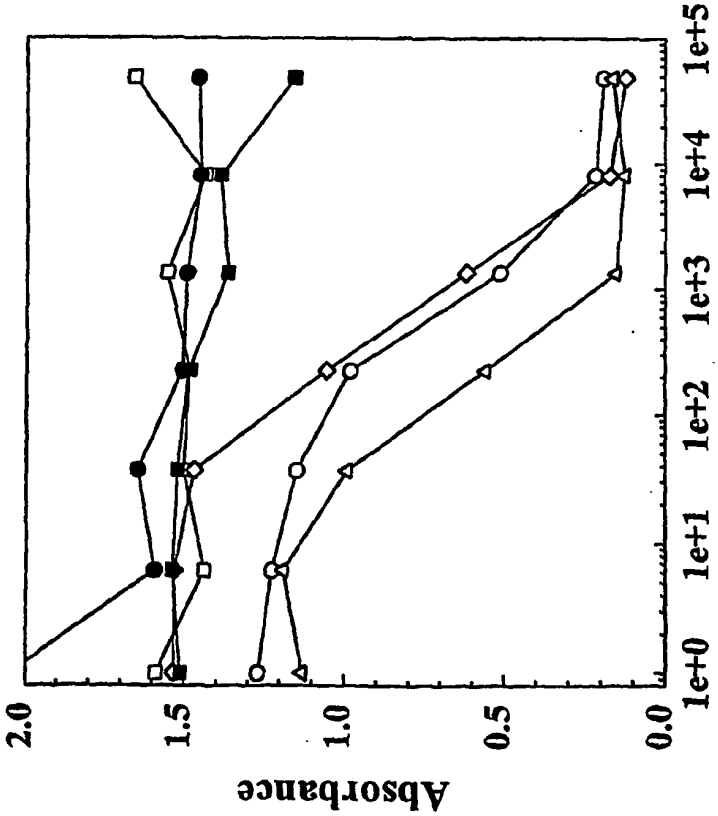


FIG. 50A

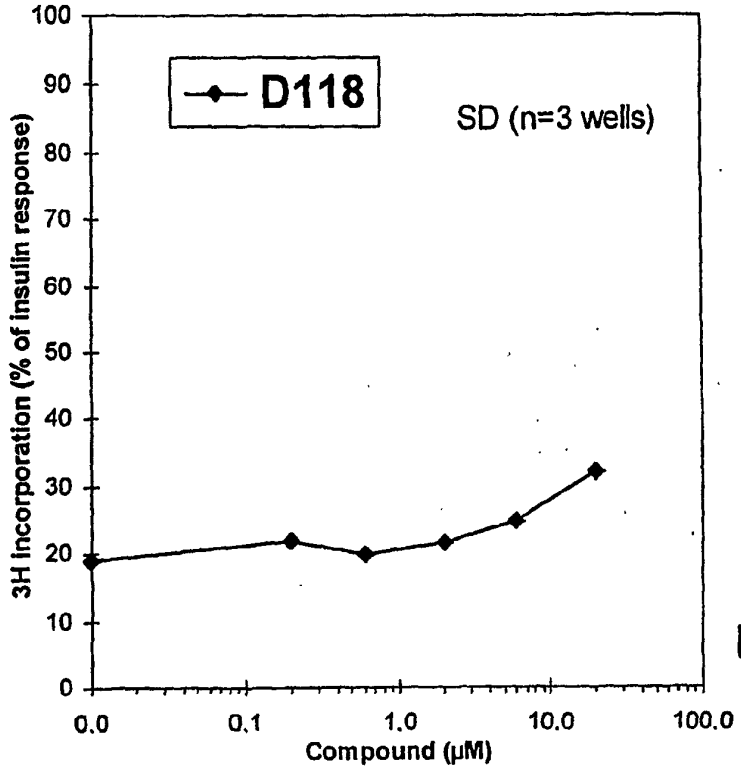


FIG. 49C

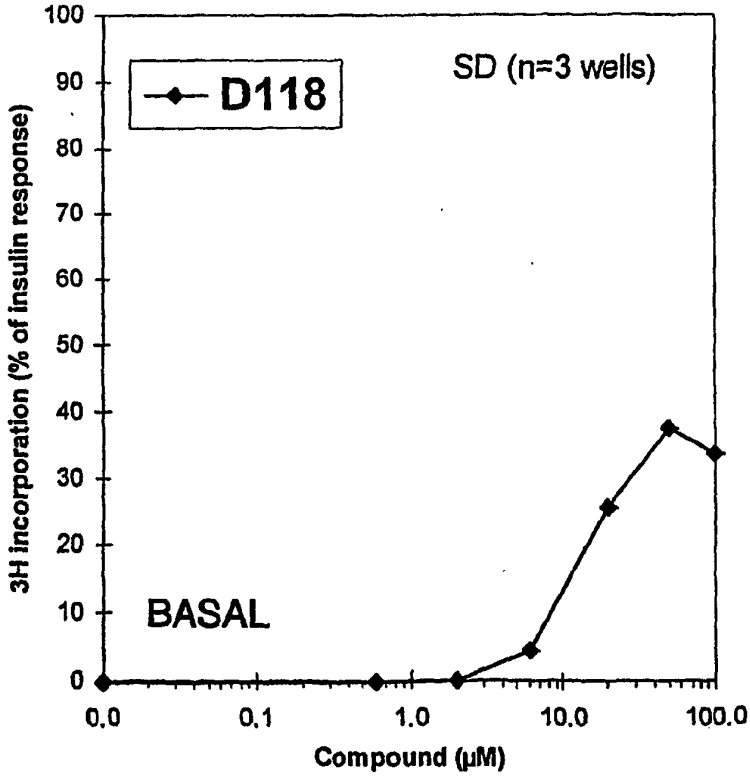


FIG. 49D

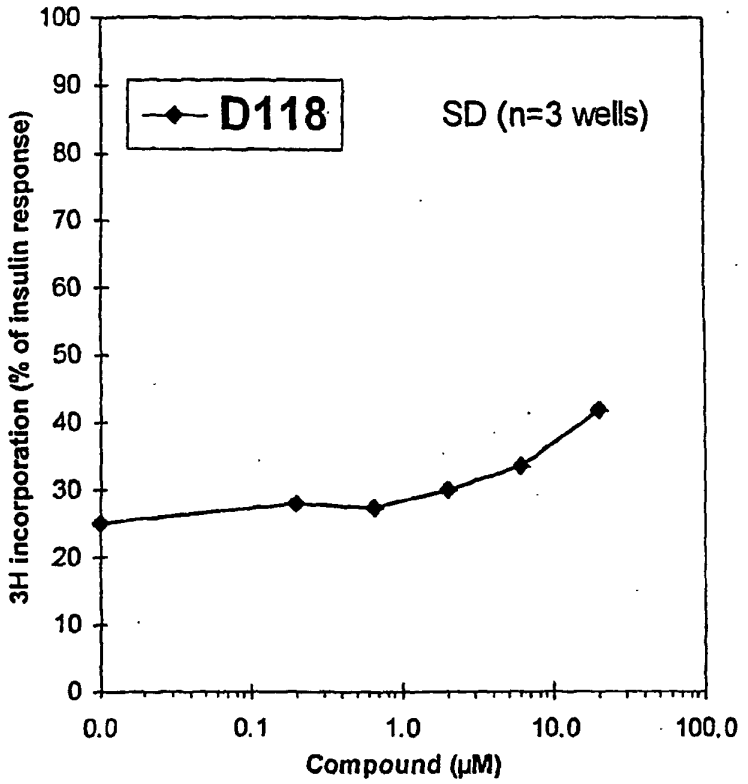


FIG. 49A

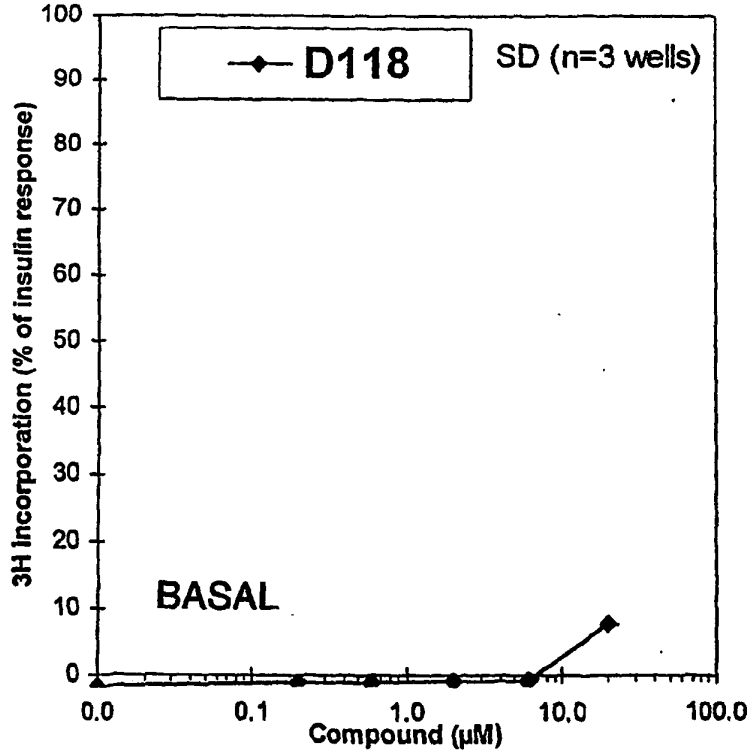


FIG. 49B

Group 4 and 6: Miscellaneous Motif 10		Target Found	IR	IGF
D10	LGPLLRWGSEVCGVWPDLCE	3	++	0
A2	GRVALWGPVWPRWWFMSRPV	1	++	+
F2	SMFVAGSDRWPGYGVLADWL	1	++	++
E8	VRGFQGGTVWPGYEWLRNAA	1	++	?
A4	WPGYLFFEEALQDWRGSTED	1	0	+++

Group 7: Formula 4 Motif		Target Found	IR	IGF
B6	ACSSFFVKGPEGFLQCLGSI	1	0	++
F8	HLCVLEELFWGASLFGYCSG	4	+++	+
40D6	PERGRGLRTAMQLMRRPRDWHFPHSLFWGAPPPLSG	1	0	0

Group 8: Non-Aligning Miscellaneous Sequences

FIG. 48-2

Group 2: Formula 6 Motif		Target Found	IR	IGF
20A4*	EIEAEWGRVRCLVYGRCVGG	13	+++	0
D8	WLDQEWAWVQCEVYGRGCPS	3	+++	?

Group 3: Formula 2 Motif		Target Found	IR	IGF
20E2	*DYKD*FYDAIDQLVRGSARAGGTRD	1	+	++++
20C11	*DYKD*DRAFYNGLRDLVGAVYGAWD	1	+	++++
20A12	*DYKD*RLFYCGIQALGANLGYSGCV	1	+	++++
C6	*DYKD*FYSALWGLCGVTGCG	1	+	+++
A6	RGQSDAFYSGLWALIGLSDG	1	+	+++
40H4	RYFPFGGFYGNLDVLRWLRPYVASPRWGHWRPGGSLGKQPT	1	+	0

Group 5: Miscellaneous Motif 10		Target Found	IR	IGF
D9-2	PFGFGGRWWGIPRMWWYRNS	1	++	++
H4	WWWGGRNRWWLERWGLGGER	1	+	+

FIG. 48-1

Gr up 1 (Formula 1 Motif)		Target Found	IR	IGF
20D3*	IGGQGQHQDGNFYDWFVEALA	18	+	++++
20F1	VFWNCRSQQLDFYEWFEQAA	16	+	++++
G3	RGGGTFYEWFESALRKHGAG	8	+	+++
20H1	RVAGAISAPGLVSNKQDGLFYSWFRE	5	+	+++
20D1*	VLQARHGCDSVSDCFYEWFA	4	+	++++
D2	DPERMQSDVGFYEWFRAAVG	3	+	+++
B8	WSALLSVMDTGFYAWFDDAV	2	++	++++
C4	DIGSDGHGRRWDSFYRWFEM	2	+	+++
A8	IGGSFVEFYGWFNDQV	2	+	+++
E7	GHSWALVRHVDRLFYEWFDL	1	++	+++
C8	LPAGGAQGFAVRGFYEWFES	1	+	+++
H8	RDKPTDQEEQNWSFYEWFRH	1	+	+++
E2	SRDQTNFTFNSAGFYGWFER	1	+	+++
B12	GAFYRWFHEALVGSERVPDV	1	+	+++
D10-2	RIGGGWARSEGFYEWFVREL	1	+	++
G8	RMFYEWFWSQMGAGPTEGSA	1	+	++
H3	HEAFYDWFSALVDGGYELMG	1	+	++
3G11	FYGWFSRQLSLTPRDDWGLP	1	+	++
F4	GVGTLTMSSDAFYTWFV	1	+	++
E7-2	LGTSAGQGVGHRAFYQWFQS	1	+	+
40G11	<--ETLESHYVVPQ----------AALDRLFYSWFS	3	+	++++
40B2	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID	2	+	++
40B12	RMGLQALAHYRKSA-----GPIFLSSGSVIKGSEGDPFYAWFRLQ	1	+	++

FIG. 47

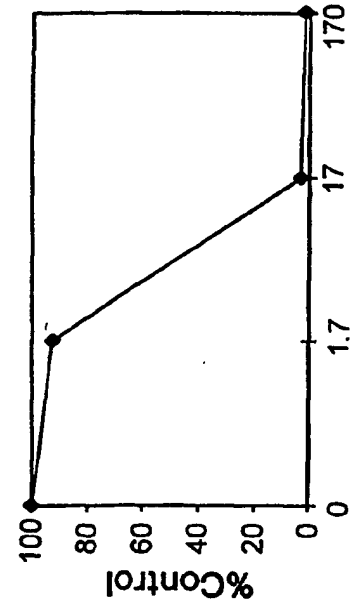


FIG. 46B

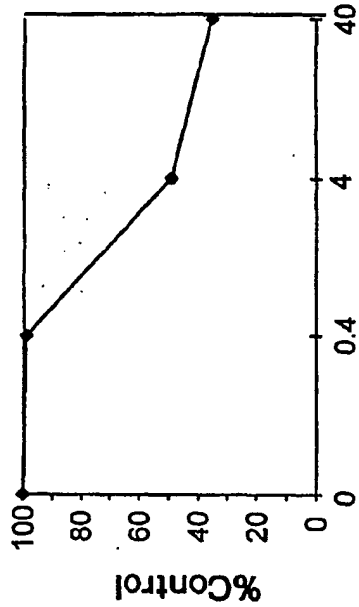


FIG. 46D

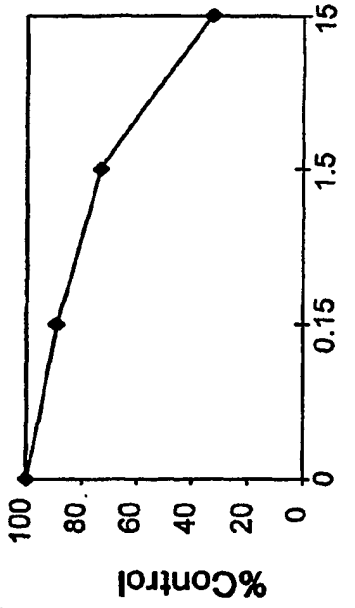


FIG. 46A

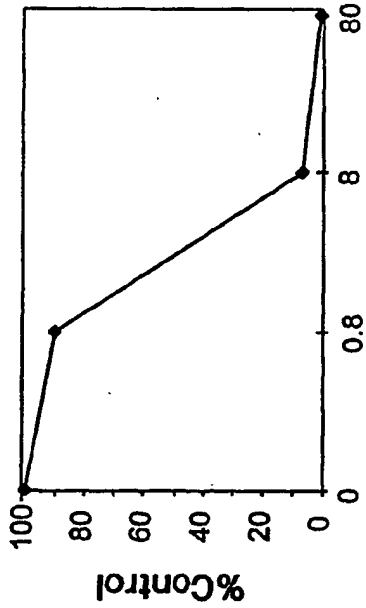


FIG. 46C

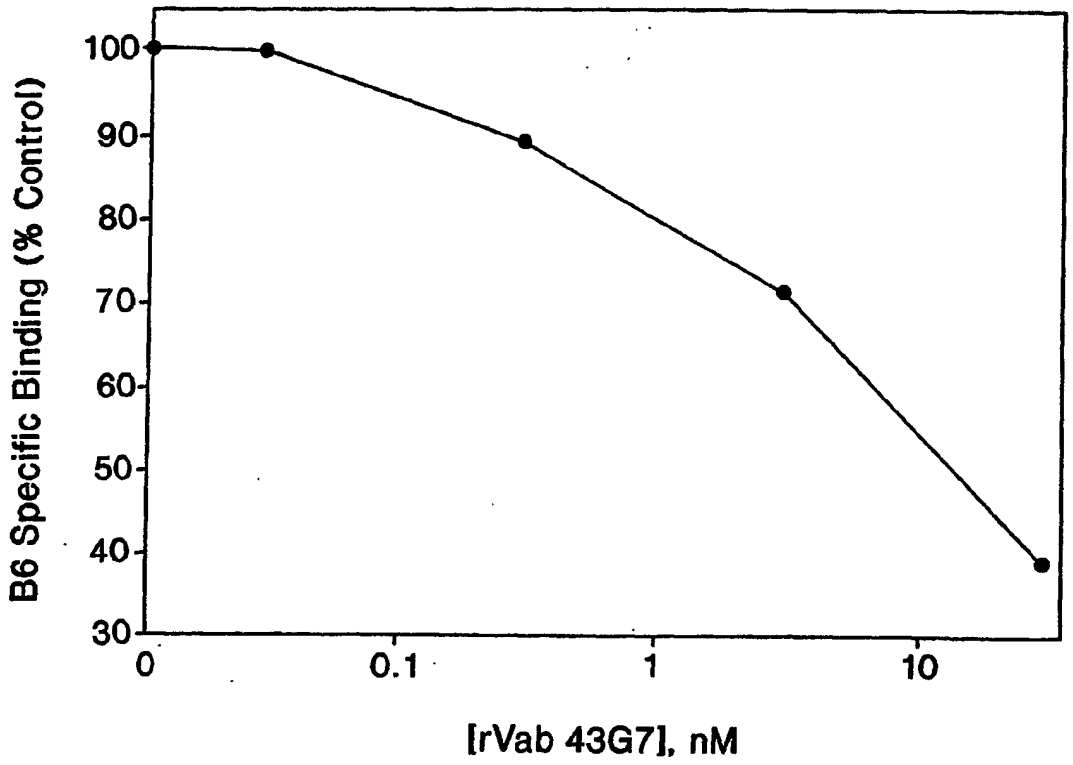

B6 Specific Binding (% Control)
100
90
80
70
60
50
40
30
0
0.1
1
10
[rVab 43G7], nM

FIG. 45

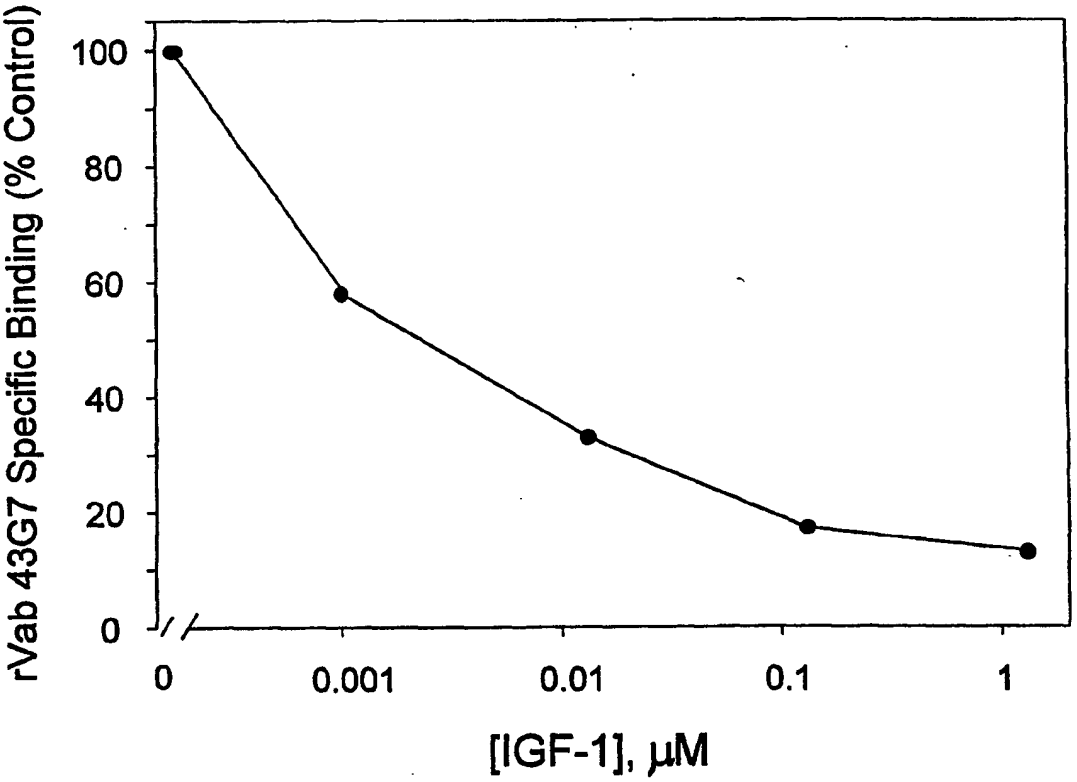

rVab 43G7 Specific Binding (% Control)
100
80
60
40
20
0
0
0.001
0.01
0.1
1
[IGF-1], µM

FIG. 44

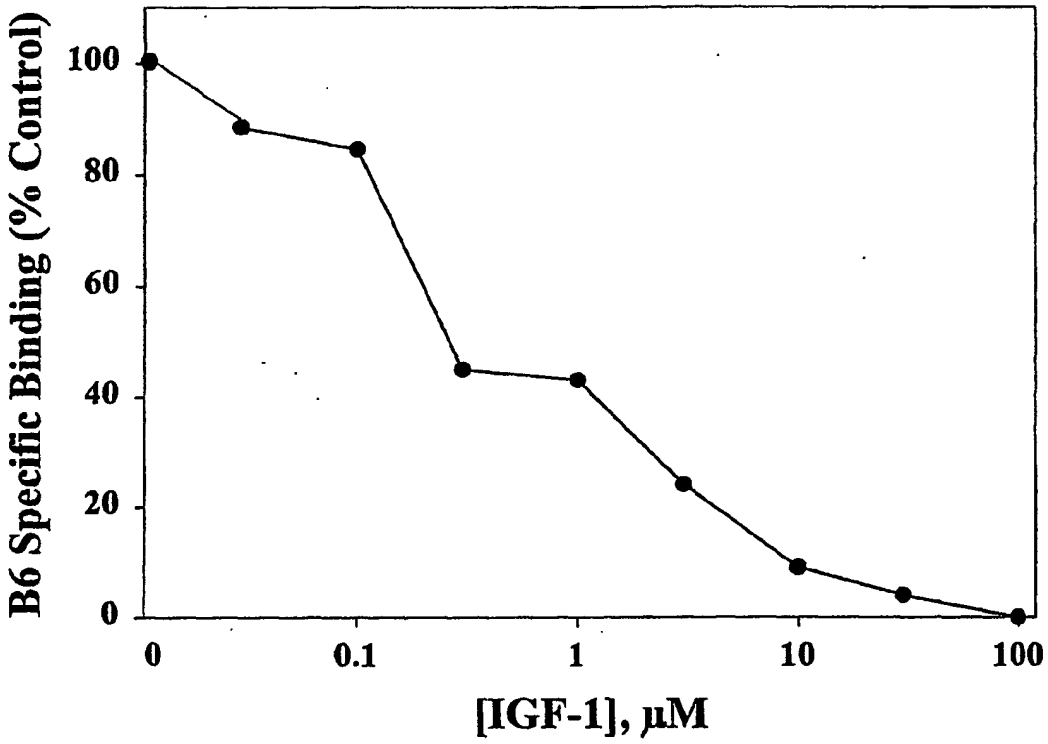

B6 Specific Binding (% Control)
100
80
60
40
20
0
0
0.1
1
10
100
[IGF-1], µM

FIG. 43

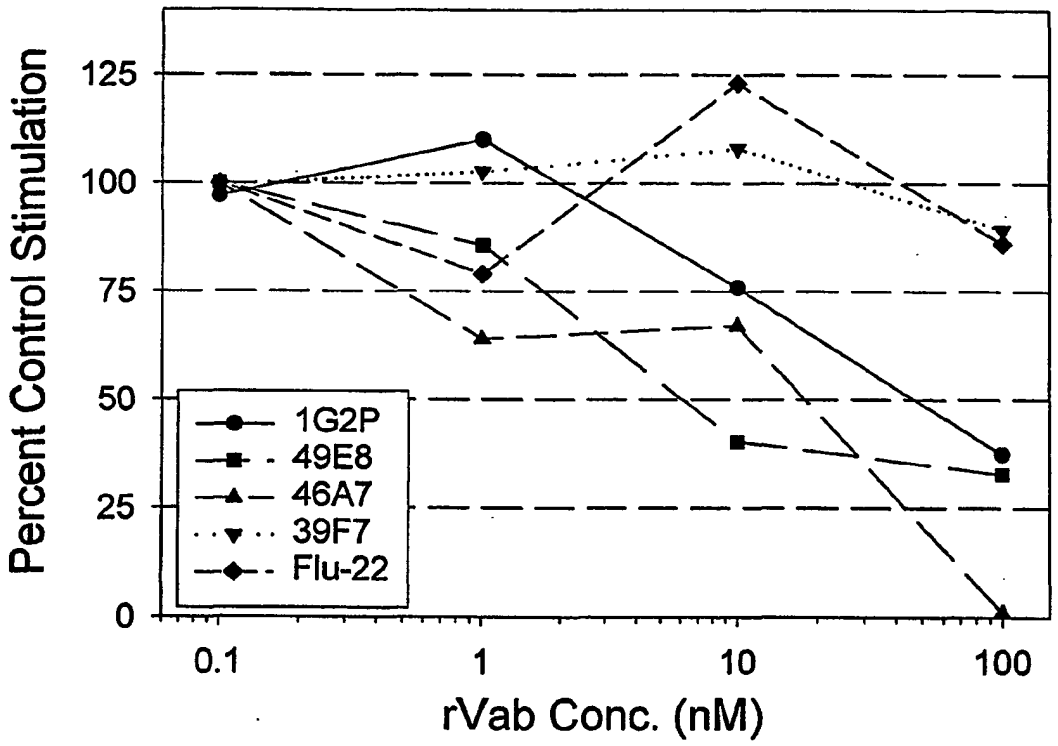

Percent Control Stimulation
125
100
75
50
25
0
0.1
1
10
100
rVab Conc. (nM)
1G2P
49E8
46A7
39F7
Flu-22

FIG. 42

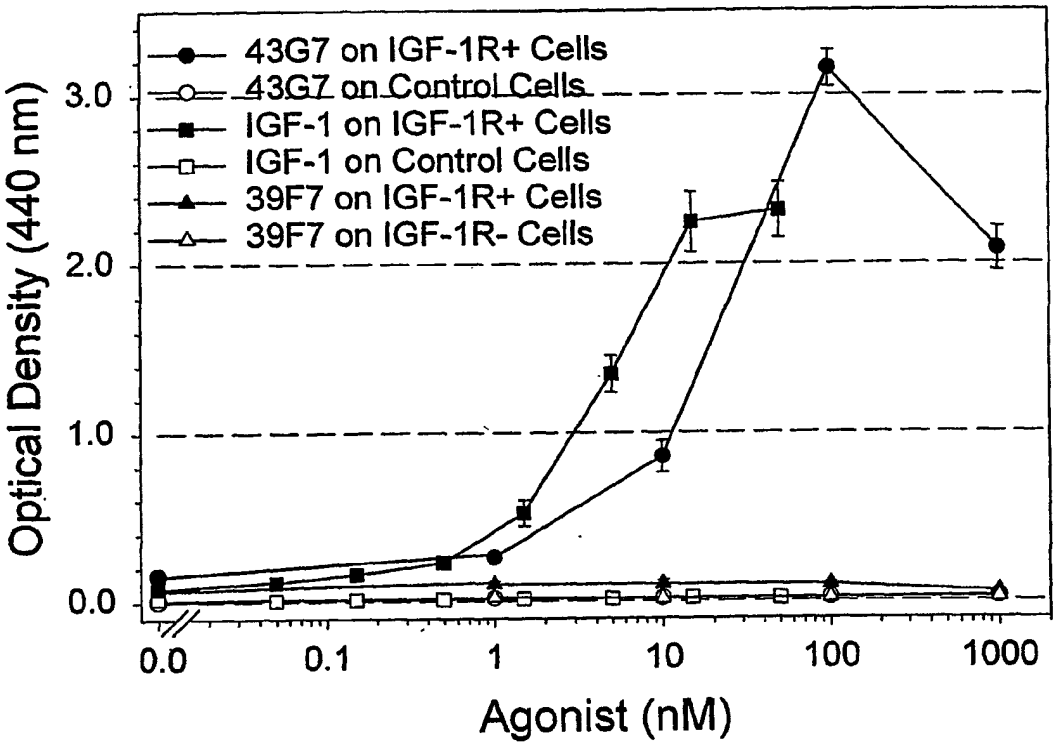

43G7 on IGF-1R+ Cells
43G7 on Control Cells
IGF-1 on IGF-1R+ Cells
IGF-1 on Control Cells
39F7 on IGF-1R+ Cells
39F7 on IGF-1R- Cells
Optical Density (440 nm)
3.0
2.0
1.0
0.0
0.0
0.1
1
10
100
1000
Agonist (nM)

FIG. 41

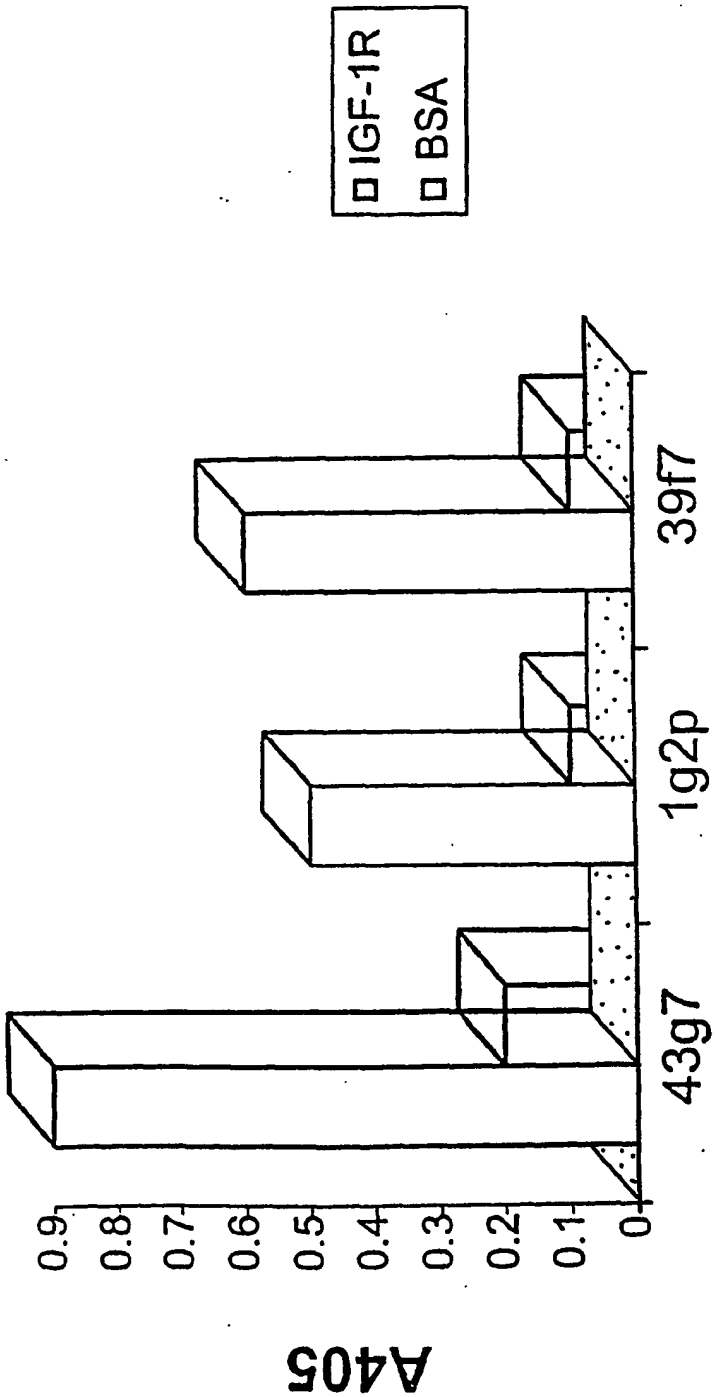


FIG. 40

```
GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGCAGTCTG GGGGAGGCTT GGTACAGCCT  60
                    Q   M   Q   L   V   Q   S   G   G   G   L   V   Q   P

GGGGGGTCCC TGAGACTCTC CTGTGCAGGC TCTGGATTCA CCTTCAGTAG CTATGCTATG 120
G   G   S   L   R   L   S   C   A   G   S   G   F   T   F   S   S   Y   A   M

CACTGGGTTC GCCAGGCTCC AGGAAAAGGT CTGGAGTGGG TATCAGCTAT TGGTACTGGT 180
H   W   V   R   Q   A   P   G   K   G   L   E   W   V   S   A   I   G   T   G

GGTGGCACAT ACTATGCAGA CTCCGTGAAG GGCCGATTCA CCATCTCCAG AGACAATGCC 240
G   G   T   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R   D   N   A

AAGAACTCCT TGTATCTTCA AATGAACAGC CTGAGAGCCG AGGACATGGC TGTGTATTAC 300
K   N   S   L   Y   L   Q   M   N   S   L   R   A   E   D   M   A   V   Y   Y

TGTGCAAGAG AGGGCGAGCT CGGGGTGACC TCCTTCTGGG GTCAAGGAAC TCTGGTCACC 360
C   A   R   E   G   E   L   G   V   T   S   F   W   G   Q   G   T   L   V   T

GTCTCCTCAG GTGGAGGCGG TTCAGGCGGA GGTGGCTCTG GCGGTGGCGG ATCCGACATC 420
V   S   S   G   G   G   G   S   G   G   G   G   S   G   G   G   S   D   I

CAGATGACCC AGTCTCCATC CTCCCTGTCT GCATCTGTAG GAGACAGAGT CACCATCACT 480
Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V   T   I   T

TGCCGGGCGA GTCAGGGCAT TAGCAATTAT TTAGCCTGGT ATCAGCAGAA ACCAGGGAAA 540
C   R   A   S   Q   G   I   S   N   Y   L   A   W   Y   Q   Q   K   P   G   K

GTTCCTAAGC TCCTGATCTA TGCTGCATCC ACTTTGCAAT CAGGGGTCCC ATCTCGGTTC 600
V   P   K   L   L   I   Y   A   A   S   T   L   Q   S   G   V   P   S   R   F

AGTGGCAGTG GATCTGGGAC AGATTTCACT CTCACCATCA GCAGCCTGCA GCCTGAAGAT 660
S   G   S   G   S   G   T   D   F   T   L   T   I   S   S   L   Q   P   E   D

GTTGCAACTT ATTACTGTCA AAAGTATAAC AGTGCCCCTT GGACGTTCGG CCAAGGGACC 720
V   A   T   Y   Y   C   Q   K   Y   N   S   A   P   W   T   F   G   Q   G   T

AAGGTGGAAA TCAAAGCGGC CGC                                          743
K   V   E   I   K
```

FIG. 39

```
GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGCAGTCTG GGGGAGGCTT GGTACAGCCT     60
                    Q   M   Q   L   V   Q   S   G   G   G   L   V   Q   P

GGGGGGTCCC TGAGACTCTC CTGTGCAGGC TCTGGATTCA CCTTCAGTAG CTATGCTATG    120
G   G   S   L   R   L   S   C   A   G   S   G   F   T   F   S   S   Y   A   M

CACTGGGTTC GCCAGGCTCC AGGAAAAGGT CTGGAGTGGG TATCAGCTAT TGGTACTGGT    180
H   W   V   R   Q   A   P   G   K   G   L   E   W   V   S   A   I   G   T   G

GGTGGCACAT ACTATGCAGA CTCCGTGAAG GGCCGATTCA CCATCTCCAG AGACAATGCC    240
G   G   T   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R   D   N   A

AAGAACTCCT TGTATCTTCA AATGAACAGC CTGAGAGCCG AGGACATGGC TGTGTATTAC    300
K   N   S   L   Y   L   Q   M   N   S   L   R   A   E   D   M   A   V   Y   Y

TGTGCAAGAT GGGGGCACGT CGGCTTGTGG GTTGCGGACG TCTATTGGGG TCAAGGAACT    360
C   A   R   W   G   H   V   G   L   W   V   A   D   V   Y   W   G   Q   G   T

CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA    420
L   V   T   V   S   S   G   G   G   G   S   G   G   G   G   S   G   G   G

TCCGACATCC AGATGACCCA GTCTCCATCC TCCCTGTCTG CATCTGTAGG AGACAGAGTC    480
S   D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V

ACCATCACTT GCCGGGCGAG TCAGGGCATT AGCAATTATT TAGCCTGGTA TCAGCAGAAA    540
T   I   T   C   R   A   S   Q   G   I   S   N   Y   L   A   W   Y   Q   Q   K

CCAGGGAAAG TTCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAATC AGGGGTCCCA    600
P   G   K   V   P   K   L   L   I   Y   A   A   S   T   L   Q   S   G   V   P

TCTCGGTTCA GTGGCAGTGG ATCTGGGACA GATTTCACTC TCACCATCAG CAGCCTGCAG    660
S   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S   S   L   Q

CCTGAAGATG TTGCAACTTA TTACTGTCAA AAGTATAACA GTGCCCCTTA CACTTTTGGC    720
P   E   D   V   A   T   Y   Y   C   Q   K   Y   N   S   A   P   Y   T   F   G

CAGGGGACCA AGCTGGAGAT CAAAGCGGCC GC                                  752
Q   G   T   K   L   E   I   K
```

FIG. 38

```
GCCCAGCCGG CCATGGCCCA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTACAGCCT        60
                Q  V  Q  L  V  E  S  G  G  G  L  V  Q  P

GGGGGATCCC TGAGACTCTC CTGTGCAGCC TCTGGATTCA CCTTCAGTAA CAGTGACATG       120
G  G  S  L  R  L  S  C  A  A  S  G  F  T  F  S  N  S  D  M

AACTGGGTCC ATCAGGCTCC AGGAAAGGGG CTGGAGTGGG TATCGGGTGT TAGTTGGAAT       180
N  W  V  H  Q  A  P  G  K  G  L  E  W  V  S  G  V  S  W  N

GGCAGTAGGA CGCACTATGC AGACTCTGTG AAGGGCCGAT TCATCATCTC CAGAGACAAT       240
G  S  R  T  H  Y  A  D  S  V  K  G  R  F  I  I  S  R  D  N

TCCAGGAACA CCCTGTATCT GCAAACGAAT AGCCTGAGGG CCGAGGACAC GGCTGTGTAT       300
S  R  N  T  L  Y  L  Q  T  N  S  L  R  A  E  D  T  A  V  Y

TACTGTGTGA GAACCGATGG CGAGTGGTAC GGGGCCTGGG GTCAAGGAAC TCTGGTCACC       360
Y  C  V  R  T  D  G  E  W  Y  G  A  W  G  Q  G  T  L  V  T

GTCTCCTCAG GTGGAGGCGG TTCAGGCGGA GGTGGCTCTG GCGGTGGCGG ATCCGCCATC       420
V  S  S  G  G  G  G  S  G  G  G  G  S  G  G  G  S  A  I

CAGATGACCC AGTCTCCATC CTCCCTGTCT GCATCTGTAG GAGACAGAGT CACCATCACT       480
Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T  I  T

TGCCGGGCAA GTCAGGGCAT TAGAAATGAT TTAGGCTGGT ATCAGCAGAA ACCAGGGAAA       540
C  R  A  S  Q  G  I  R  N  D  L  G  W  Y  Q  Q  K  P  G  K

GCCCCTAAGC TCCGGATCTA TGCTGCATCC AGTTTACAAA GTGGGGTCCC ATCAAGGTTC       600
A  P  K  L  R  I  Y  A  A  S  S  L  Q  S  G  V  P  S  R  F

AGCGGCAGTG GATCTGGCAC AGATTTCACT CTCACCATCA GCAGCCTGCA GCCTGAAGAT       660
S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P  E  D

TTTGCAACTT ATTACTGTCT ACAAGATTAC AATTACCCTC TCACTTTCGG CGGAGGGACC       720
F  A  T  Y  Y  C  L  Q  D  Y  N  Y  P  L  T  F  G  G  G  T

AAGGTGGAGA TCAAAGCGGC CGC                                               743
K  V  E  I  K
```

FIG. 37

```
GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT    60
                    Q  M  Q  L  V  E  S  G  G  G  L  V  K  P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG   120
G  G  S  L  R  L  S  C  A  A  S  G  F  T  F  S  N  A  W  M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA   180
S  W  V  R  Q  A  P  G  K  G  L  E  W  V  G  R  I  K  S  K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA   240
T  D  G  G  T  T  D  Y  A  A  P  V  K  G  R  F  T  I  S  R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC   300
D  D  S  K  N  T  L  Y  L  Q  M  N  S  L  K  T  E  D  T  A

GTGTATTACT GTACCACACC GGGCTGGTAT GGGGCCGAGG ATAAGTGGGG TCAAGGAACT   360
V  Y  Y  C  T  T  P  G  W  Y  G  A  E  D  K  W  G  Q  G  T

CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA   420
L  V  T  V  S  S  G  G  G  G  S  G  G  G  G  S  G  G  G

TCCGACATCC AGATGACCCA GTCTCCATCC TCCCTGTCTG CATCTGTAGG AGACAGAGTC   480
S  D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V

ACCATCACTT GCCGGGCGAG TCAGGGCATT AGCAATTATT TAGCCTGGTA TCAGCAGAAA   540
T  I  T  C  R  A  S  Q  G  I  S  N  Y  L  A  W  Y  Q  Q  K

CCAGGGAAAG TTCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAATC AGGGGTCCCA   600
P  G  K  V  P  K  L  L  I  Y  A  A  S  T  L  Q  S  G  V  P

TCTCGGTTCA GTGGCAGTGG ATCTGGGACA GATTTCACTC TCACCATCAG CAGCCTGCAG   660
S  R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q

CCTGAAGATG TTGCAACTTA TTACTGTCAA AAGTATAACA GTGCCCCTTT CACTTTCGGC   720
P  E  D  V  A  T  Y  Y  C  Q  K  Y  N  S  A  P  F  T  F  G

CCTGGGACCA AAGTGGATAT CAAAGCGGCC GC                                 752
P  G  T  K  V  D  I  K
```

FIG. 36

```
GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT   60
                  Q   M   Q   L   V   E   S   G   G   G   L   V   K   P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG  120
G   G   S   L   R   L   S   C   A   A   S   G   F   T   F   S   N   A   W   M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA  180
S   W   V   R   Q   A   P   G   K   G   L   E   W   V   G   R   I   K   S   K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA  240
T   D   G   G   T   T   D   Y   A   A   P   V   K   G   R   F   T   I   S   R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC  300
D   D   S   K   N   T   L   Y   L   Q   M   N   S   L   K   T   E   D   T   A

GTGTATTACT GTACCACATG GGGCTCCGTC GACACGGACA ACTACGCCAG GTTTTGGGGT  360
V   Y   Y   C   T   T   W   G   S   V   D   T   D   N   Y   A   R   F   W   G

CAAGGAACTC TGGTCACCGT CTCCTCAGGT GGAGGCGGTT CAGGCGGAGG TGGCTCTGGC  420
Q   G   T   L   V   T   V   S   S   G   G   G   G   S   G   G   G   G   S   G

GGTGGCGGAT CCGACATCCA GATGACCCAG TCTCCATCCT CCCTGTCTGC ATCTGTAGGA  480
G   G   G   S   D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G

GACAGAGTCA CCATCACTTG CCGGGCGAGT CAGGGCATTA GCAATTATTT AGCCTGGTAT  540
D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y   L   A   W   Y

CAGCAGAAAC CAGGGAAAGT TCCTAAGCTC CTGATCTATG CTGCATCCAC TTTGCAATCA  600
Q   Q   K   P   G   K   V   P   K   L   L   I   Y   A   A   S   T   L   Q   S

GGGGTCCCAT CTCGGTTCAG TGGCAGTGGA TCTGGGACAG ATTTCACTCT CACCATCAGC  660
G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S

AGCCTGCAGC CTGAAGATGT TGCAACTTAT TACTGTCAAA AGTATAACAG TGCCCCTCTC  720
S   L   Q   P   E   D   V   A   T   Y   Y   C   Q   K   Y   N   S   A   P   L

ACTTTCGGCG GAGGGACCAA GGTGGAGATC AAAGCGGCCG C                      761
T   F   G   G   G   T   K   V   E   I   K
```

FIG. 35

```
GCCCAGCCGG CCATGGCCCA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT    60
                  Q   V  Q  L   V  E  S  G   G  G  L   V  K  P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG   120
G  G  S  L   R  L  S   C  A  A   S  G  F  T   F  S  N   A  W  M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA   180
S  W  V  R   Q  A  P   G  K  G   L  E  W  V   G  R  I   K  S  K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA   240
T  D  G  G   T  T  D   Y  A  A   P  V  K  G   R  F  T   I  S  R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC   300
D  D  S  K   N  T  L   Y  L  Q   M  N  S  L   K  T  E   D  T  A

GTGTATTACT GTACCACAGT TGCGTTGTCT GCCGACCGTG GGATGTGGGG TCAAGGAACT   360
V  Y  Y  C   T  T  V   A  L  S   A  D  R  G   M  W  G   Q  G  T

CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA   420
L  V  T  V   S  S  G   G  G  G   S  G  G  G   G  S  G   G  G  G

TCCGATGTTG TGATGACTCA GTCTCCACTC TCCCTGCCCG TCACCCTTGG ACAGCCGGCC   480
S  D  V  V   M  T  Q   S  P  L   S  L  P  V   T  L  G   Q  P  A

TCCATCTCCT GCAGGTCTAG TCAAAGCCTC GTATACAGTG ATGGAAACAC CTACTTGAAT   540
S  I  S  C   R  S  S   Q  S  L   V  Y  S  D   G  N  T   Y  L  N

TGGTTTCAGC AGAGGCCAGG CCAATCTCCA AGGCGCCTAA TTTATAAGGT TTCTAACCGG   600
W  F  Q  Q   R  P  G   Q  S  P   R  R  L  I   Y  K  V   S  N  R

GACTCTGGGG TCCCAGACAG ATTCAGCGGC AGTGGGTCAG GCACTGATTT CACACTGAAA   660
D  S  G  V   P  D  R   F  S  G   S  G  S  G   T  D  F   T  L  K

ATCAGCAGGG TGGAGGCTGA GGATGTTGGG GTTTATTACT GCATGCAAGG TACACACTGG   720
I  S  R  V   E  A  E   D  V  G   V  Y  Y  C   M  Q  G   T  H  W

CCTTACACTT TTGGCCAGGG GACCAAGCTG GAGATCAAAG CGGCCGC                 767
P  Y  T  F   G  Q  G   T  K  L   E  I  K
```

FIG. 34

```
                   DPL16 (=v3s1)+
Lambda 3 genes:   /    +v318    /        /  JL2/3 for DPL16+v3s1+v318        /  NotI /  PCR primer site    /
                  Ser           His        ValValPheGlyGlyGlyThrLysLeuThrValLeu
                  NNN---------CAT          GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG
                GGNNN---------G          TACACCATAAGCCGCCTCCCTGGTTCGACTGGCAGGATCGCCGGCGTCACACTCAGGTTTTCTAAAGC

Lambda 3 gene:    /DPL23 (=VL3.1)/      for DPL23+VL3.1
                  Ser           Ala        GTG---   continued as for JL2/3
                  NNN---------GCA        GTCAC---
                GGNNN---------C

Lambda 1 genes:   / DPL2+DPL3    /
                  Gln           Gly      for DPL2+DPL3
                  NNN---------GGT          GTG---   continued as for JL2/3
                GGNNN---------C          CACAC---

Lambda 2 gene:    /    DPL11     /
                  Gln           Leu      for DPL11
                  NNN---------CTC          GTG---   continued as for JL2/3
                GGNNN---------G          AGCAC---
```

FIG. 33B

```
                    /     VH gene        / CDR H3 /            JH4b gene            /              G4S linker
  Ec RI /  SfiI      Glu      Arg          6x-12x  TrpGlyGluGlyThrLeuValThrValSerSerGlyGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySer
CCGGAATTCGGCCCAGCCGGCCNNN------NGA         NNB---NNBTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCC
GGCCTTAAGCCGGGTCGGCCGGNNN------N          CTNNV---NNVACCCCGGTCCCTTGGGACCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACCGCCTA
           3' end for 40 VH genes                                                                                  BamHI

CCGGAATTCGGCCCAGCCGGCCNNN------NAA         NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N          TTNNM---NNM
            3' end for 7 VH genes: DP31, DP33, DP39, DP40, f1p1, DP47 and DP49                 BOLD = IN DGI COLLECTION

CCGGAATTCGGCCCAGCCGGCCNNN------NCA         NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N          GTNNM---NNM
            3' end for 4 VH genes: DP2, DP3, DP5 and DP38

All kappa genes:             /          JK1 gene          /  NotI /  PCR primer site    /
  Asp         Pro             TrpThrPheGlyGlnGlyThrLysValGluIleLys
  NNN---------CCT             TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG
GGNNN---------G              GAACCTGCAAGCCGGTTCCCTGGTTCCACCTTTAGTTTCGCCGGCGTCACACTCAGGTTTTCTAAAGC
 (except VK L20)
                             /          JK2 gene          /
                              TyrThrPheGlyGlnGlyThrLysLeuGluIleLys
                              TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA continued as for JK1
                             GAATGTGAAAACCGGTCCCCTGGTTCGACCTCTAGTTT

                             /          JK3 gene          /
                              PheThrPheGlyProGlyThrLysValAspIleLys
                              TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA continued as for JK1
                             GAAAGTGAAAGCCGGGACCCTGGTTTCACCTATAGTTT

                             /          JK4 gene          /
                              LeuThrPheGlyGlyGlyThrLysValGluIleLys
                              CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA continued as for JK1
                             GAGAGTGAAAGCCGCCTCCCTGGTTCCACCTCTAGTTT
```

FIG. 33A

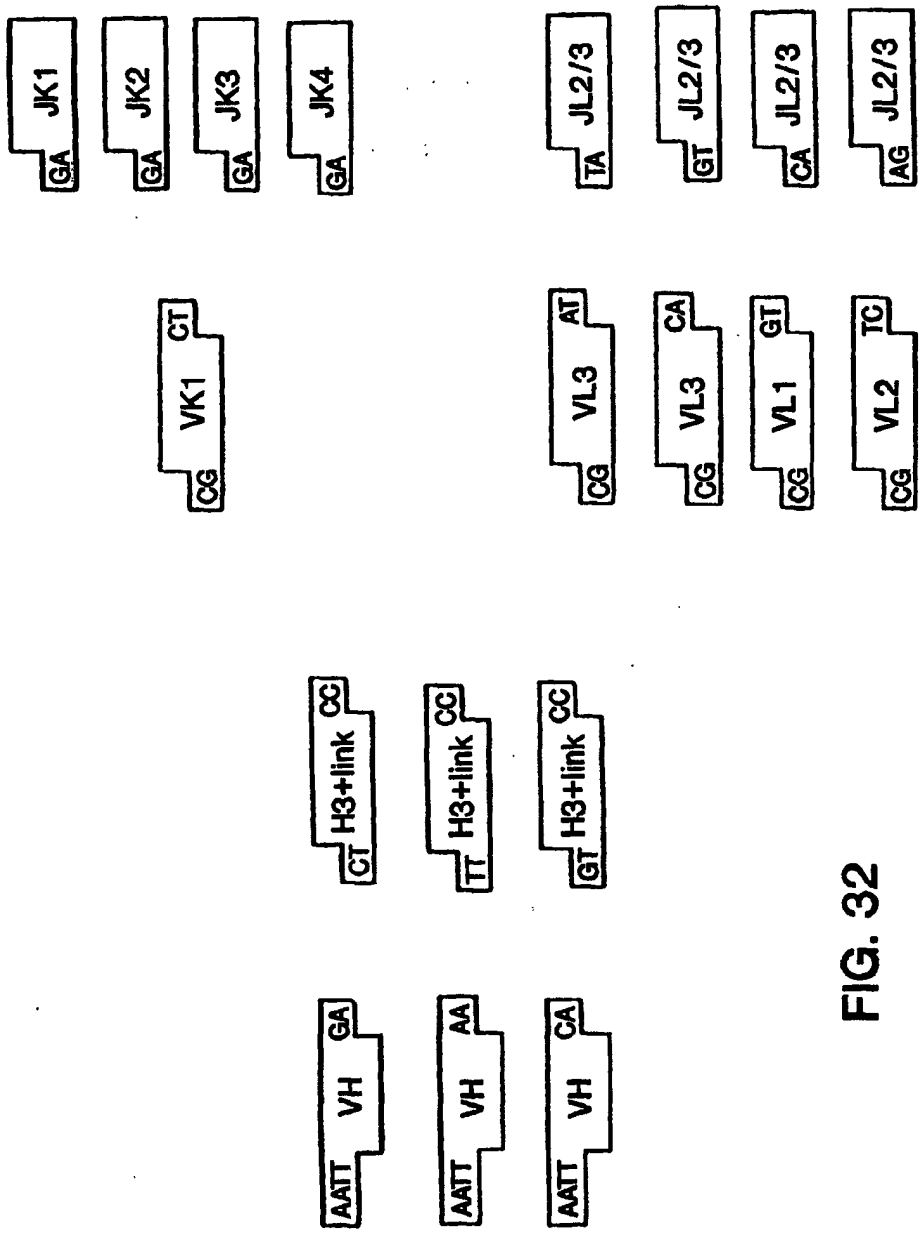

JK1
GA
JK2
GA
JK3
GA
JK4
GA
JL2/3
TA
JL2/3
GT
JL2/3
CA
JL2/3
AG
VK1
CG
CT
VL3
CG
AT
VL3
CG
CA
VL1
CG
GT
VL2
CG
TC
H3+link
CT
CC
H3+link
TT
CC
H3+link
GT
CC
VH
AATT
GA
VH
AATT
AA
VH
AATT
CA

FIG. 32

VH Gene Sequences	Lambda and Kappa Gene Sequences
DP-1	DPK11
DP-10	DPK15
DP-12	DPK18
DP-14	DPK2/L14+
DP-15	DPK3/L11+
DP-2	DPK4
DP-21	DPK6
DP-25	DPK8/Vd+
DP-29	DPL23
DP-30	HK101
DP-31	L22+
DP-32	L23/L23a
DP-33	LFVK431
DP-35	VA++
DP-38	
DP-39	
DP-40	
DP-42	
DP-44	
DP-45	
DP-46	
DP-47	
DP-5	
DP-50	
DP-51	
DP-52	
DP-53	
DP-54	
DP-59	
DP-63	
DP-66	
DP-67	
DP-68	
DP-69	
DP-7	
DP-70	
DP-71	
DP-73	
DP-74	
DP-8	
hv1263	
VHD26	

FIG. 31

Genomic rVab Library

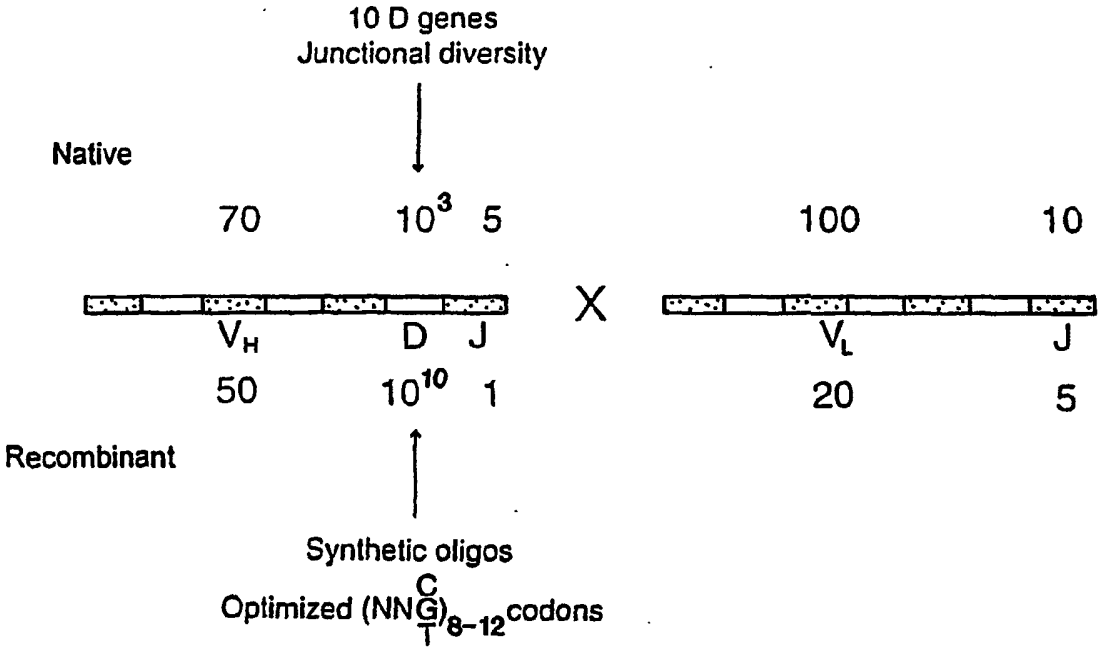


FIG. 30

Name	Sequence	#Found	Ratio	IGF Inh.	GHR+
H5:	LCQSLGVTYPGWLAGWCA	-	1.2	-	2.6
2C3:	VCQRLGGTFPGWLVGVCR	-	1.1	-	1.1
JBA5:	LCQSWGVRI-GWLAGLCP	19	~24.0	~45%	1.2
E2A12:	LCQSLGFTDLDWLACWFE	10	~17.5	~54	1.1
E1A11:	VCQSLGITDLGLCAGWGA	1	16.4	50	1.0
E4B10:	LCQSLGLTHPGFEAWLCA	5	~11.7	~50	1.2
E4C10:	LCQNFGVTDPGCFYGWFA	1	9.9	~51	0.7
E4A9:	LCQSSGLSFLGCL-GWWA	14	~8.5	~65	1.0
JBB6:	PCQRLGDTHLCWLAGWFA	6	~8.3	~65	1.1
E1F9	PCQSLGLTCSGWFEGWGA	1	8.3	68	1.2
E4G7:	QWQSLGVTCPGSWAELCA	1	6.0	50	1.3
E4A11:	LWQSVGIKYPGGLAGWLA	1	5.8	67	1.4
E1B9:	LCQSLGVTYWEGLAWLCA	3	5.5	60	1.1
E4A12:	VCQGLGVECPGWFAGWWA	3	~5.3	~55	1.2
E4F11:	LCQGWGIRI-GWLVGRCM	1	2.7	58	1.1
E1D3:	LCQSLGVTYPGWLAGGCA	1	2.0*	-	1.0

FIG. 29

A6S-4-H5	DYKD	HHSPGNEHGY**NFYDWFV**LqVA	AAA	24	19	123
A6S-4-E4	DYKD	ERSAAGFREG**NFYDWFV**AqVN	AAA	32	27	120
A6S-4-F5	DYKD	GSQHSGREPH**NFYDWFV**AqVG	AAA	28	24	120
A6S-4-D4	DYKD	IARMRETFqP**NFYDWFV**DQLA	AAA	21	18	118
A6S-4-C6	DYKD	RLDRSSTSGV**NFYDWFV**AqVG	AAA	28	25	116
A6S-4-D3	DYKD	GLRSEqGNRL**NFYDWFV**AqIA	AAA	23	20	116
A6S-4-F2	DYKD	SVIqTRqDET**NFYDWFV**?AMS	AAA	26	23	115
A6S-4-A5	DYKD	VEVqRHIRKD**NFYDWFV**KQID	AAA	22	19	115
A6S-4-C3	DYKD	VTMLDKGAqD**NFYDWFV**REVA	AAA	24	21	114
A6S-4-F3	DYKD	HNSSSPMRTG**NFYDWFV**qELR	AAA	30	26	113
A6S-4-B4	DYKD	ERSPRPALAS**NFYDWFV**QqVV	AAA	21	19	113
A6S-4-B6	DYKD	SDARqAGLQE**NFYDWFV**SQVR	AAA	26	23	113
A6S-4-B1	DYKD	RHERGKEGPG**NFYDWFV**SqVV	AAA	21	19	112
A6S-4-G4	DYKD	SALSGPVqPI**NFYDWFV**TGM	AAA	30	26	112
A6S-4-A6	DYKD	HVEHMAVGDG**NFYDWFV**VqLR	AAA	23	21	111
A6S-4-F4	DYKD	VGHSGVPPYP**NFYDWFV**MqVS	AAA	24	22	110
A6S-4-D6	DYKD	LGAAETWDGI**NFYDWFV**KqVS	AAA	24	22	110
A6S-4-E6	DYKD	RSSGGLLSqG**NFYDWFV**SQLE	AAA	26	24	109
A6S-4-A3	DYKD	LAINDLVTHK**NFYDWFV**DqLR	AAA	20	18	109
A6S-4-E3	DYKD	RGMTGMVGRG**NFYDWFV**GqLR	AAA	23	21	109
A6S-4-A2	DYKD	IGGQGqHQDG**NFYDWFV**EALA	AAA	22	20	107
A6S-4-B2	DYKD	qSVDLSRPDS**NFYDWFV**EVLS	AAA	22	21	105
A6S-4-H2	DYKD	VTFTSAVFHE**NFYDWFV**RqVS	AAA	20	19	104
A6S-4-D1	DYKD	SNPSRqDASV**NFYDWFV**REVA	AAA	22	22	103
A6S-4-H1	DYKD	IVAGARHSEV**NFYDWFV**IqVR	AAA	18	18	102
A6S-4-E2	DYKD	?DGQSVSSKG**NFYDWFV**QqMT	AAA	25	25	101
A6S-4-G1	DYKD	AELVGAGVRG**NFYDWFV**DqLV	AAA	16	16	101
A6S-4-G2	DHKD	SAGHHMPRES**NFYDWFV**DqVV	AAA	24	25	99
A6S-4-A1	DYKD	DSSRLWLGER**NFYDWFV**AqIS	AAA	12	17	68

FIG. 28-2

Clone:		Binding Ratios: Target	E-Tag	% Max
A6S-1-C5	DYKD RIHNQTERGGNFYDWFVHqLV AAA	7	27	26
A6S-1-G3	DYKD VATVHVGGGMNFYDWFVAqVG AAA	5	19	26
A6S-1-A2	DYKD KDPVTVSQGRNFYDWFVVqIQ AAA	5	20	25
A6S-1-D5	DYKD RVGSGMEDLGNFYDWFVRQAq AAA	5	25	20
A6S-1-H4	DYKD HKSWTTMSPLNFYDWFVAqVE AAA	3	18	17
A6S-2-F2	DYKD LAMSVASRPANFYDWFVAqIV AAA	30	35	86
A6S-2-D2	DYKD RAERGSMRDSNFYDWFVqQLP AAA	30	36	83
A6S-2-E3	DYKD VqEGLSGMEGNFYDWFVDQLF AAA	28	36	78
A6S-2-H2	DYKD RGqRESDSGTNFYDWFVGAIR AAA	28	40	70
A6S-2-A3	DYKD SRAPYGSTAGNFYDWFVqAVS AAA	25	37	68
A6S-2-H1	DYKD RVGIqVDPHTNFYDWFVIQLT AAA	27	42	64
A6S-2-F1	DYKD VGqVGRYVRSNFYDWFVQqAM AAA	8	30	27
A6S-2-G1	DYKD RPqLVESGSKNFYDWFVqVVR AAA	8	30	27
A6S-2-B2	DYKD EMYGDTSERVNFYDWFVSALq AAA	5	30	17
A6S-2-A1	DYKD LSSRGRVTMRNFYDWFVAqVV AAA	3	31	10
A6S-3-E10	DYKD RVREKLPRPENFYDWFVNqIH AAA	22	23	96
A6S-3-G2	DYKD TWMWEERKqDNFYDWFVGQLK AAA	20	21	95
A6S-3-E5	DYKD RYRGERHDGRNFYDWFVEqVN AAA	19	21	90
A6S-3-H2	DYKD qGAEGRLSEGNFYDWFVQAVS AAA	19	21	90
A6S-3-H9	DYKD YSIEVqDWNENFYDWFVSQLG AAA	20	23	87
A6S-3-G3	DYKD PRLHMGSDMGDFYDWFVVqIA AAA	18	21	86
A6S-3-F8	DYKD GRGqGLKRPDNFYDWFVAAAK AAA	20	25	80
A6S-3-G10	DYKD GAVGLAEAGPNFYDWFVSqVq AAA	19	24	79
A6S-3-H1	DYQD PASNKNSLAENFYDWFVqQTR AAA	23	30	77
A6S-3-E6	DYKD DARDHGVWVMSNFYDWFVAqVS AAA	5	20	25
A6S-3-D9	DYKD SLQGADFqQGNFYDWFVSELA AAA	4	17	24
A6S-3-E3	DYKD RPSLPEVRPGNFYDWFVqSVR AAA	4	19	21
A6S-3-H8	DYKD NPTSVqQYGVNFYDWFVNVLS AAA	4	20	20
A6S-3-G4	DYKD CADPGACSSLNFYDWFVqMRG AAA	4	21	19
A6S-3-B10	DYKD YDqDPPYWGLNFYDWFVREVA AAA	3	16	19
A6S-3-C1	DYKD RPVIGGGGTRNFYDWFVAqMI AAA	3	17	18
A6S-4-G5	DYKD QEVTRTRDDKNFYDWFVSqIF AAA	26	18	144
A6S-4-D2	DYKD PPYRSSRLGENFYDWFVMqVR AAA	26	19	143
A6S-4-F6	DYKD LKGSSqPLSVNFYDWFVQQIK AAA	24	17	142
A6S-4-H4	DYKD PRMVEKPSEDNFYDWFVTqLS AAA	28	20	141
A6S-4-C1	DYKD CWARPCGDAANFYDWFVqQAS AAA	22	16	141
A6S-4-G3	DYKD GAQAIREIHHNFYDWFVAqVT AAA	29	21	139
A6S-4-H3	DYKD GRGDQRHETTNFYDWFVRELq AAA	28	20	137
A6S-4-H6	DYKD GSIAQLIMRANFYDWFVEqTN AAA	24	18	130
A6S-4-G6	DYKD RLMGGIAEPqNFYDWFVREVA AAA	25	20	126

FIG. 28-1

GACTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCTGGGTCGTATCTCTGACG
D Y K D D D D K Y R G M L V L G R I S D

GTGCTGGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTT**AA**

G A G K V A S E P P A R I G Q K V F A V **N**

CTTCTACGACTGGTTCGTTGCGGCCGCA 96 nt
F Y D W F V A A A

FIG. 25A

CTACAAAGACGACGATGACAAG**TACCGTGGTATGCTGGTTCTGGGTCGTATCTCTGACG**
GTGCTGGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTT
AACTTCTACGACTGGTTCGTTGCGGCCGCAGTGTGA 154 nt

FIG. 25B

GACTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTCG
D Y K D X X X X X X X X X X N F Y D W F

TTNNKNNKNNKNNK 21 aa
V X X X X

FIG. 26A

CTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTCGTTNNK
NNKNNKNNKGCGGCCGCAGTGTGA

FIG. 26B

H5	NH_2-D-Y-K-D-L-C-Q-S-L-G-V-T-Y-P-G-W-L-A-G-W-C-A-K-K(Biotin)-COOH
H5 Control	NH_2-D-Y-K-D-W-C-L-T-L-Q-P-L-V-W-A-S-G-G-G-Y-C-A-K-K(Biotin)-COOH
2C3-60	NH_2-D-Y-K-D-V-C-Q-R-L-G-G-T-F-P-G-W-L-V-G-V-C-R-K-K(Biotin)-COOH
H5-447	NH_2-D-Y-K-D-L-C-Q-R-L-G-V-G-W-P-G-W-L-S-G-W-C-A-K-K(Biotin)-COOH
H5-432	NH_2-D-Y-K-D-L-C-Q-S-L-G-V-T-W-P-G-W-L-A-G-W-C-A-K-K(Biotin)-COOH

FIG. 27

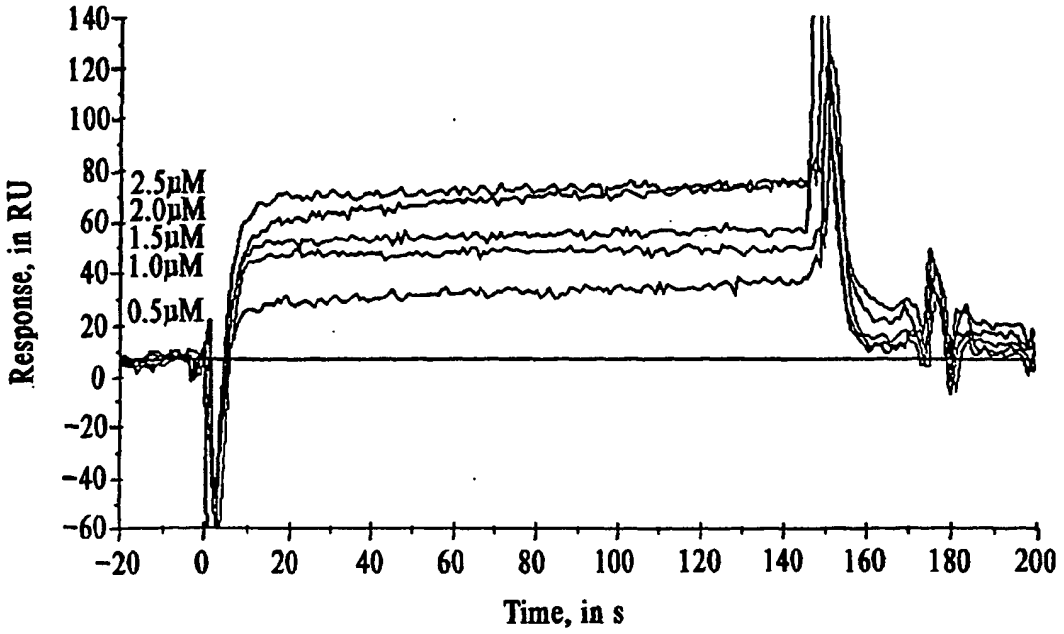


FIG. 24A

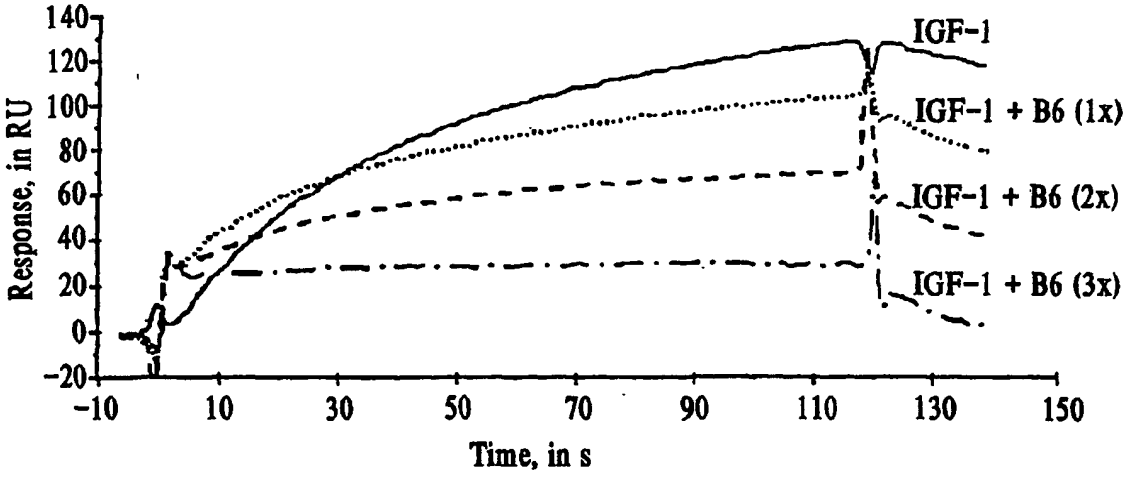


FIG. 24B

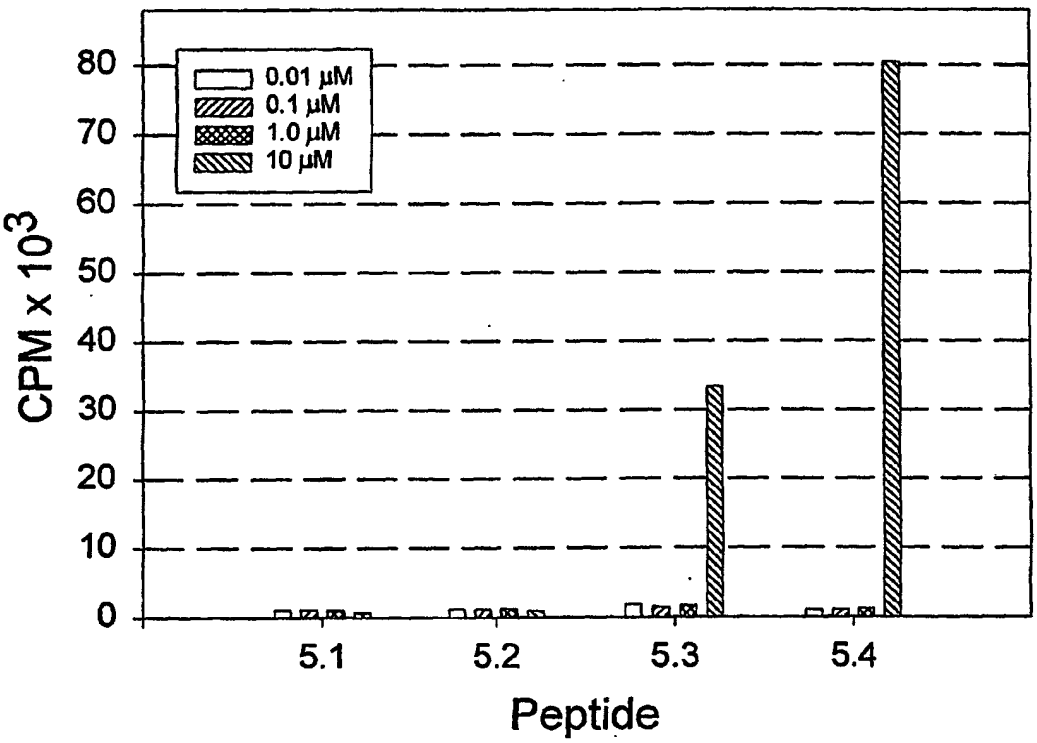

0.01 µM
0.1 µM
1.0 µM
10 µM
CPM x 10^3
80
70
60
50
40
30
20
10
0
5.1
5.2
5.3
5.4
Peptide

FIG. 23

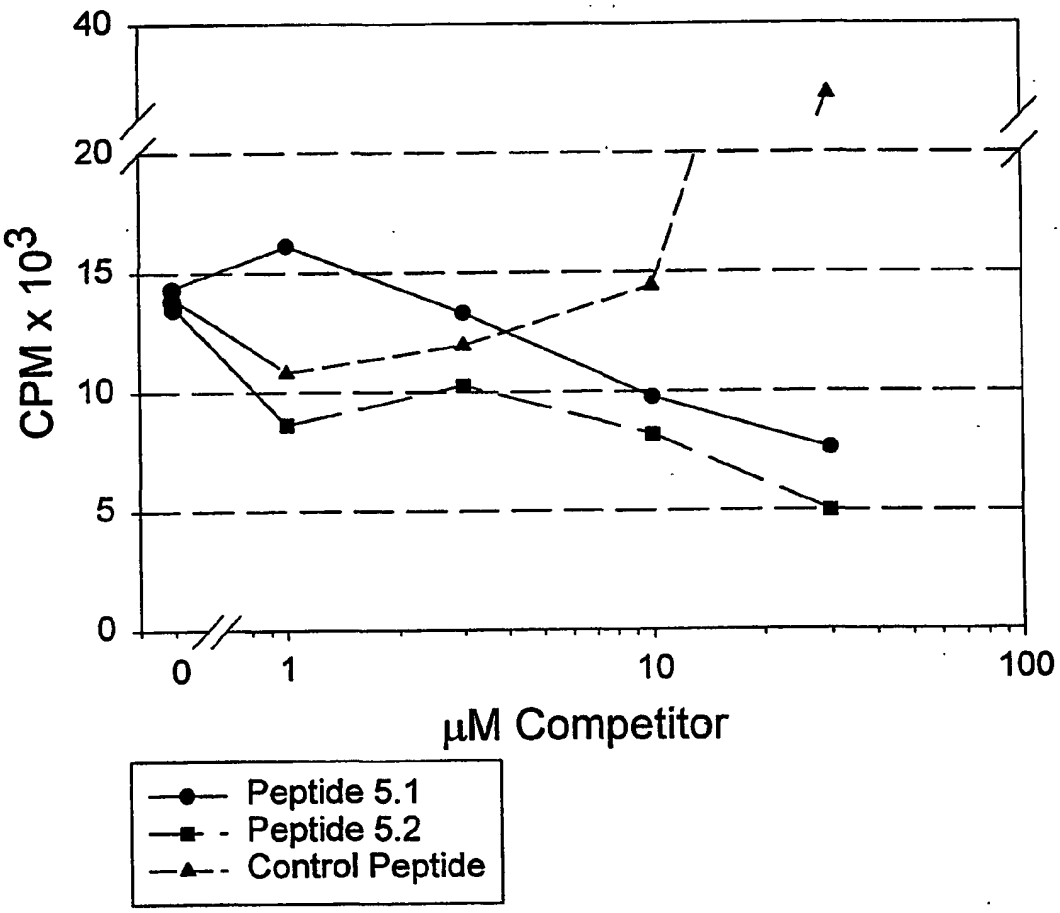

40
20
15
10
5
0
CPM x 10^3
0
1
10
100
μM Competitor
Peptide 5.1
Peptide 5.2
Control Peptide

FIG. 22

Clone B6
Peptide 5.1 (18 aa)

AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA
NTVDPFYHKLSELLREKK(biotin)

Clone F6
Peptide 5.2 (17 aa)

MLLGVLRFQILLWPFPKDCVQMKDIFYSLLASL
QMKDIFYSLLASLAAKK(biotin)

Clone D5
Peptide 5.3 (14 aa)

PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM
ADKNFYDWFMAAKK(biotin)

Clone A6
Peptide 5.4 (12 aa)

YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV
SAKNFYDWFVKK(biotin)

FIG. 21

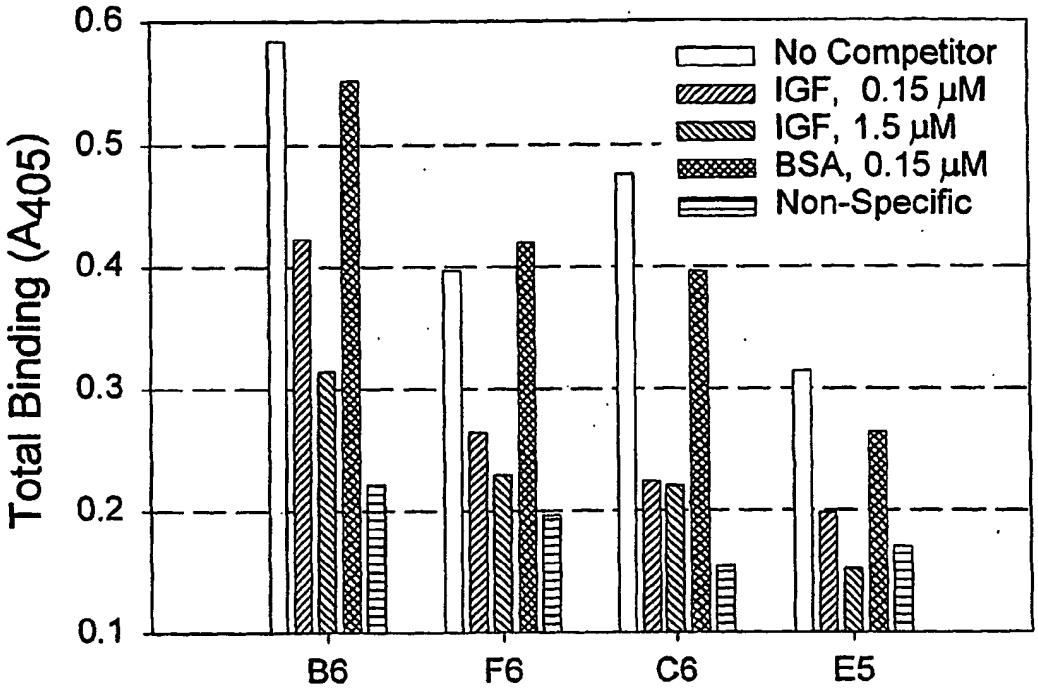


FIG. 20A

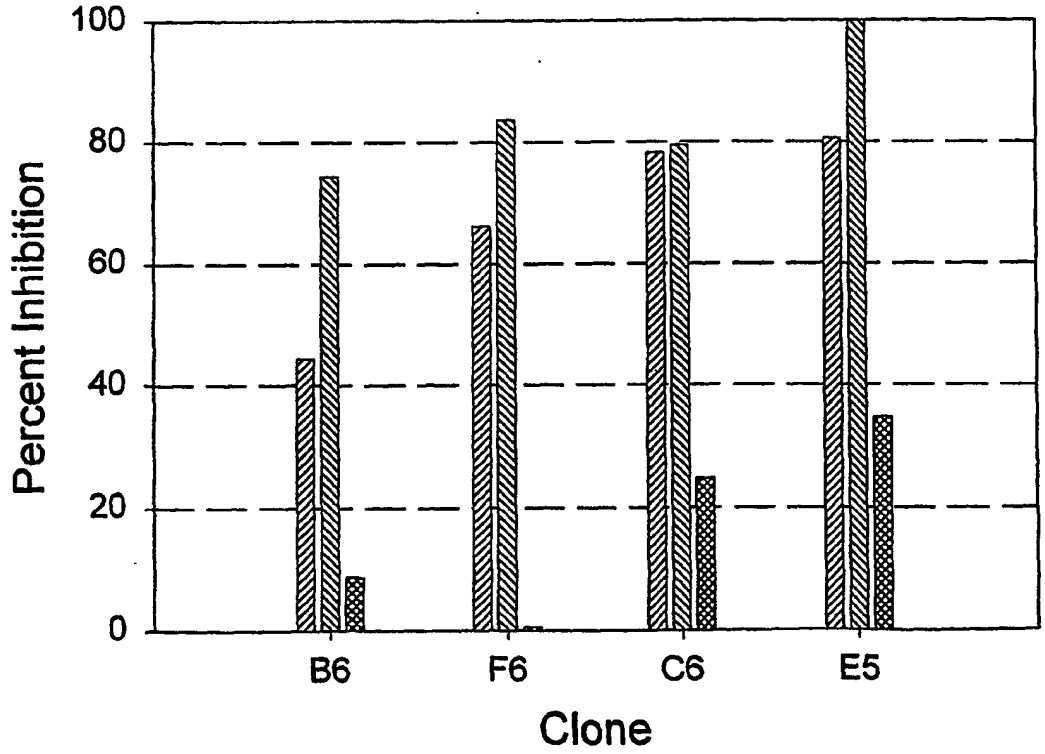


FIG. 20B

IGF-5

```
GACTACAAAG ACTCGTGGTT GAATTTTCGG TATGTTGCTG GGCGTGCTTA GGTTTCAGAT TCTTCTGTGG
D Y K D  S W L  N F R  Y V A G  R A Q  V S D  S S V A
 T T K  T R G .  I F G  H L L  G V L R  F Q I  L L W
  L Q R  L V V  E F S V  C C W  A C L  G F R F  F C G

CCGTTTCCTA AGGATTGTGT TCAGATGAAA GATATTTTTT ATTCGTTGTT GGCTAGTTTG GCGGCCGCA  139
  V S .  G L C  S D E R  Y F L  F V V  G Q F G  G R
P F P K  D C V  Q M K  D I F Y  S L L  A S L  A A A
 R F L  R I V F  R . K  I F F  I R C W  L V W  R P
```

IGF-8

```
GACTACAAAG ACGCGGTTGC GGCTGCTGTT GCTCCTTGGG GGTGATGAGC CTTTTTATGG GCTTCTCCGT
D Y K D  A V A  A A V  A O W G  . .  A  F L W  A S P Y
 T T K  T R L R  L L L  L L G  G D E P  F Y G  L L R
  L Q R  R G C  G C C C  S L G  V M S  L F H G  F S V

ATGCTGATTG GTCGTGGGTC TGCGGCCGCA  100
  A D W  S W V  C G R
H L I G  R G S  A A A
 C . L  V V G L  R P
```

IGF-G5

```
GACTACAAAG ACTGGTTGGT TTGCTTGGGT GTGATGATTA GCTTTTTCTG TTTAGGGGGT CGGTGTGGCT
D Y K D  W L V  C L G  V M I S  F F C  L G G  R C G F
 T T K  T G W F  A W V  . .  L  A F S V  Q G V  G V A
  L Q R  L V G  L L G C  D D Q  L F L  F R G S  V W L

TTTTACTCAG CGTTGGCTGC CTTGTTGTGT GCCCATAGTG CTTCTTTGGT GTGTGGTGCG GCGGCCGCA  139
  L L S  V G C  L V V C  P Q C  F F G  V W C G  G R
F Y S A  L A A  L L C  A H S A  S L V  C G A  A A A
 F T Q  R W L P  C C V  P I V  L L W C  V V R  R P
```

IGF-7

```
GACTACAAAG ACCCGGATTG GGTGTTGTAG CTGATTAGTT TGGGGTTGGA GGGGATGTAG ATTGGCTGAT
D Y K D  P D W  V L Q  L I S L  G L E  G M Q  I G . W
 T T K  T R I G  C C S  . L V  W G W R  G C R  L A D
  L Q R  P G L  G V V A  D Q F  G V G  G D V D  W L M

GGGTTTTATG CGTTTTTGAT GGCGC1GGCT GGGGCGGCCG CA  112
  V L C  V F D  G A G W  G G R
G F Y A  F L M  A L A  G A A A
 G F M  R F .  W R W L  G R P
```

FIG. 19

Class I Clones		# Clones Rnd 3	# Clones Rnd 4	Competition
B6 3x	DYKDAETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1	2	+
E5 2x	DYKDRHLTNAELGVQSPEVLSRLFPDGDIFYRALSHLVRGMGPP	2		+
B5 2x	DYKDRGGMDRQWLDVGARHRLERRSVQDNTDDFYGGLRILVDGF	2		nd
9	DYKDGPPDSFDVTEKGDMAILNVRFDPHSLDFNDQTFYFLLDSL		1	nd
G6	DYKDGGTYFRGQVAQSNESLLRVNFLQLLEALAASPPT		1	nd
12	DYKDAPFDARLSAPRFQWSPRTWXQSLSYGEWSCGSFYDCLSSI		1	nd
A5	DYKDMGSSQFQDTRPSSGQAYSHSLDSDGWGTANWIFLRALEGL		1	nd
C6	DYKDSGAAHEGNQGRERSTHLAANINDHLPGDAGIWLGYSWLS		1	+
Consensus (regular+frameshifters)	d.FY.lLsaL			
	s			
Human IGF mature	VCGDRGFYFNKPTGYGSSSR			
	23 30			
Human IGF propeptide	MSSSHLFYLALCLLTFTSSA			
	-16 -9			

Class I frameshifting clones (all in +1 frame)				
F6 2x	TTKTRG.IFGMLLGVLRFQILLWPFPKDCVQMKDIFYSLLASL	1	1	+
7 3x	TTKTRIGCCS.LVWGWRGCRLADGFYAFLMALAG		3	nd
8	TTKTRLRLLLLLGGDEPFYGLLRMLIGRGS		1	nd
G5	TTKTGWFAWV..LAFSVQGVHVAFYSALAALLCAHSASLVCGA	1		nd

Class II clones				
D5	DYKDPLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM	1		nd
A6	DYKDYRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	1		nd
R35	DYKDSGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	1		nd

human IGF mature (1-70)

GPETLCGAEL VDALQFVCGD RGFYFNKPTG YGSSSRRAPQ TGIVDECCFR SCDLRRLEMY CAPLKPAKSA

nd = no data

FIG. 18

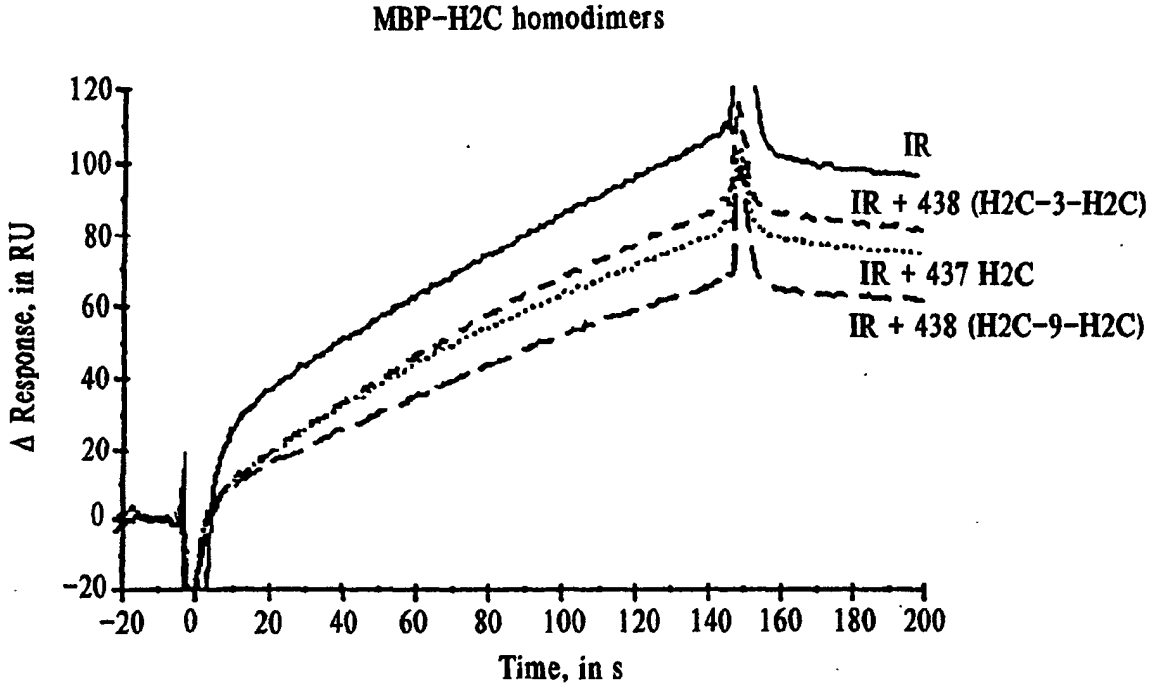

MBP-H2C homodimers
Δ Response, in RU
Time, in s
IR
IR + 438 (H2C-3-H2C)
IR + 437 H2C
IR + 438 (H2C-9-H2C)
120
100
80
60
40
20
0
-20
-20
0
20
40
60
80
100
120
140
160
180
200

FIG. 17

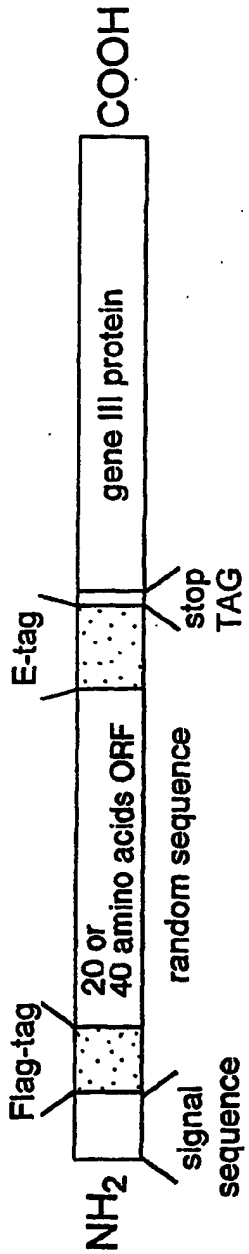

COOH
gene III protein
stop
TAG
E-tag
20 or
40 amino acids ORF
random sequence
Flag-tag
signal
sequence
NH2

FIG. 16

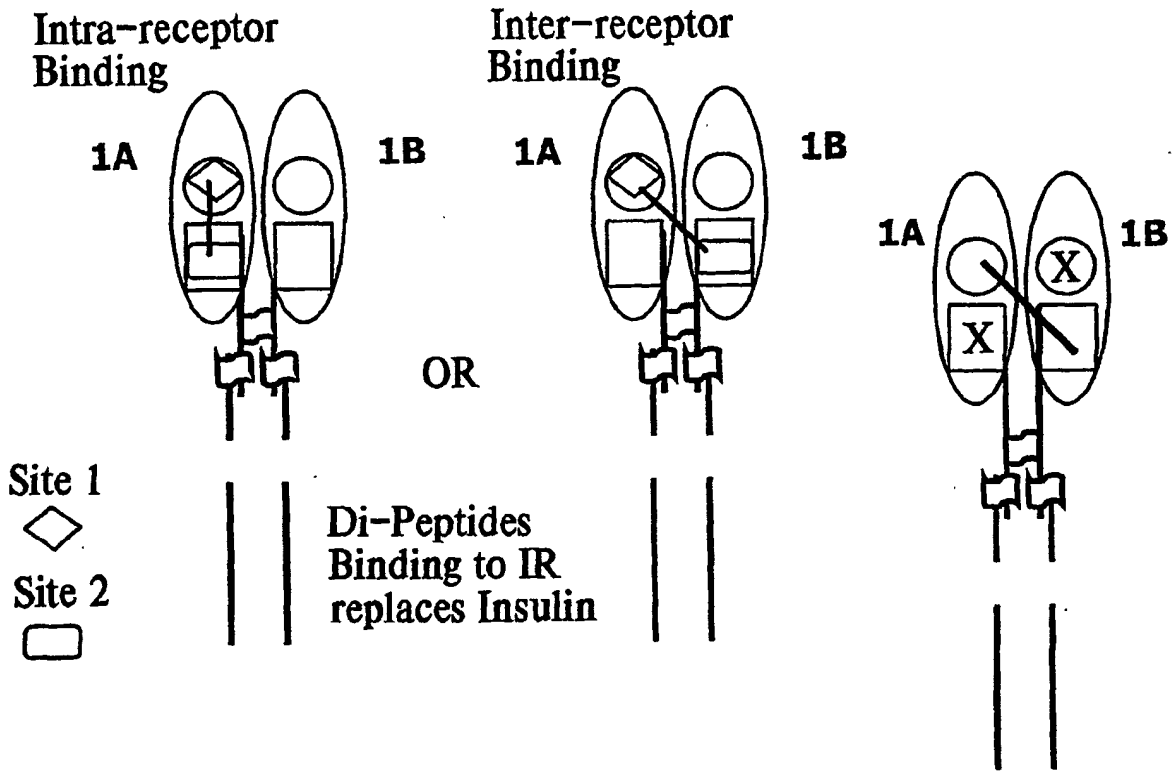

Intra-receptor Binding
1A
1B
Inter-receptor Binding
1A
1B
OR
1A
1B
X
X
Site 1
Site 2
Di-Peptides Binding to IR replaces Insulin

FIG. 15

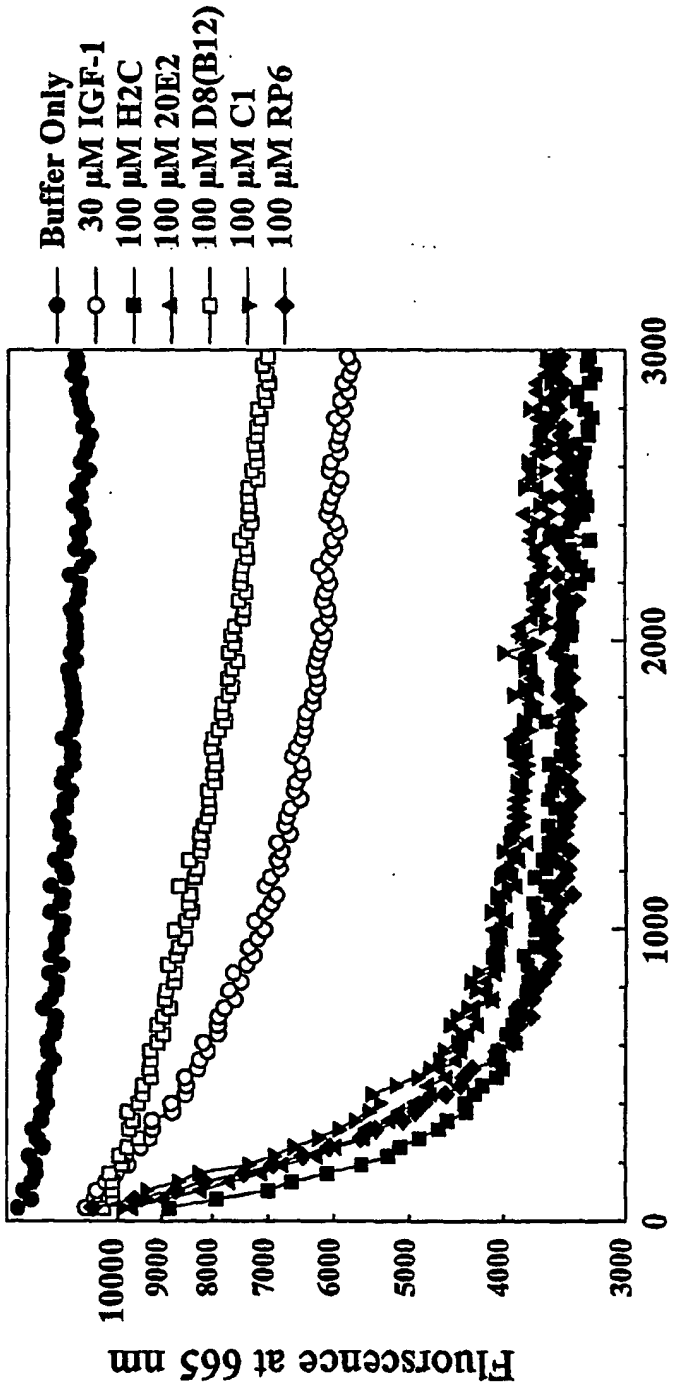

Buffer Only
30 µM IGF-1
100 µM H2C
100 µM 20E2
100 µM D8(B12)
100 µM C1
100 µM RP6
Fluorscence at 665 nm
10000
9000
8000
7000
6000
5000
4000
3000
0
1000
2000
3000
Time (s)

FIG. 14

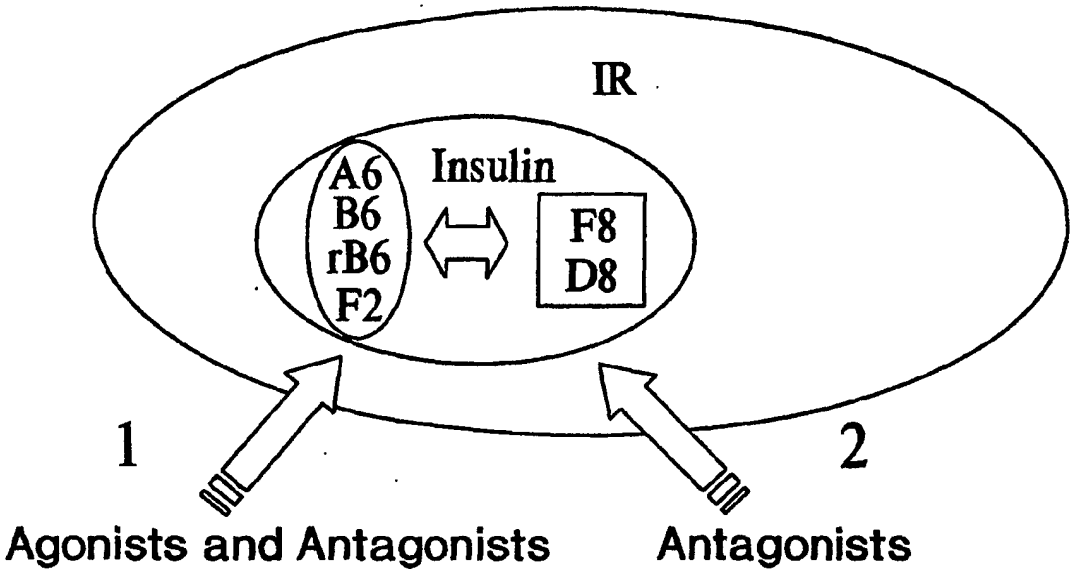
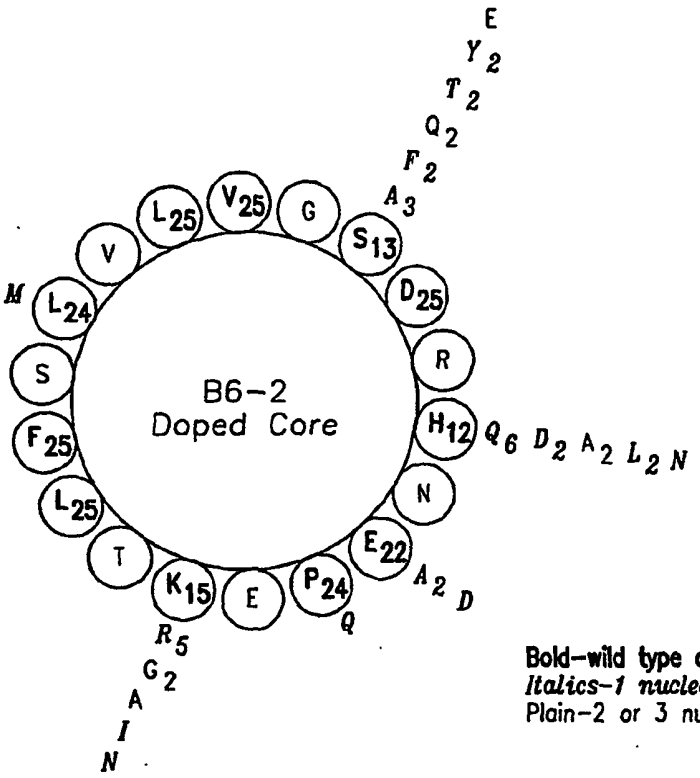

IR
Insulin
A6
B6
rB6
F2
F8
D8
1
2
Agonists and Antagonists
Antagonists

FIG. 13



Bold–wild type amino acid
Italics–1 nucleotide required to change aa
Plain–2 or 3 nucleotide required to change aa

FIG. 12

Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC_{50} Biacore	IR-IC_{50} FP_{-S175}	PO_4	Fat Cell Assay
S177	S177	B6	EHWNTVDPFYTLFEWLRESG	2.7 μM				+
S178	S178	B6	EHWNTVDPFYQYFSELLRESG	130 nM				0
S179	S179	A6	QSDSGTVHDRFYGWFRDTWAS	540 nM				++
F8-C12	S224	C-C LOOP	FQSLLEELVWGAPLFRYGTG					
S225	S225	C-C LOOP	PLCVLEELFWGASLFGYCSG					
D8-G1	S226	C-C LOOP	QLEEEWAGVQCEVYGRECPS					
S264	S264	A6	IQGWEPFYGWFDDVAQMFEE					
S257	S257	B6	RWPNFYGYFESLLTHFS					
S258	S258	B6	HYNAFYEYFQVLLACTW					
S259	S259	B6	EGWDFYSYFSGLLASVT					

FIG. 11C

Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC 50 Biacore	IR-IC50 pp-S175	PO_4	Fat Cell Assay
20-E2	D118	B6	DYKDFYDAIDQLVRGSARAGGTRDK K-biotin	250 nM		2.8 nM	+	++
C1	D112	A6	DYKDCWARPCGDAANFYDWFVDQAS KK-biotin	490 nM			-	0
D8	D123	C-C LOOP	KWLDQEWAWVQCEVYGRGCPSKK	550 nM			0	-
E8	D120	GROUP 6	KRGFQGGTVWPGYEWLRNA	370 nM			-	-
F8	D124	C-C LOOP	KHLCVLEELFWGASLFGYCSGKK	40 nM			-	0
H2C	D117	A6	FHENFYDWFVRQQVSKK	700 nM	>5 μM	5 nM	++	++
KCF9			RLYYEWFWGQLEAQGRGGLS					
KC-G2		C-C-C	CLEQGCPWVGLEVQCRGCPS		>1 μM			
KCG7		B6	FYCGLEELSWGAALFGYCSG					
NG-C2		B6	GNGDGMFYQLLSLVGRDMH					
NG-C33		A6	GHSQSCPESFYDWFAGQVSDPWWCW		2-4 μM	4.2 nM	+++	
NG-G8		B6	VEGRGLFYQLLRQLLARRQNG		>5 μM		-	
NG-G9		B6	RAMSFYDALWLGLGPKK-Biotin				-	
RP-1		A6	GSRPVFHEQFYEWFVDQLGL		1 μM		+	
RP-2		A6	RSEASFHVEFYSWFEEQLRS		1 μM		+	
RP-3		A6	GRFYGWFQDAIDQLMPWGFD		>10 μM		-	
RP-4		B6	PPWGARFYDAIEQLVFDNL		5 μM		+	
RP-5		B6	AGVNAGFYRYFSILLDWWDQGKK-Biotin		6 μM		-	
RP-6		B6+ C-C	TFYSCLASLLTGTPQPNRGPWERCRKK-Biotin				+++++	
RP-7		A6	AAVHEQFYDWFADQYKK					
RP-8		B6	QSFYDYIEELLGGEWKK		>5 μM		+	
RP-8#	S287	B6	QSFYDYIEELLGGEWEE			2.9 nM	++	
RP-9		A6	GSLDESFYDWFERQLGKK		>10 μM		+	
RP-10		B6	GSFYEALQRLVGGEQGKK		>10 μM		+	
RP-11		A6	QAPSNFYDWFVREWDKK					
RP-12		B6	DPFYQGLWEWLRESGKK					
RP-13		A6	ASGFPENFYDWFGRQLSLKK		>10 μM			
RP-14		A6	SACQFDCHENFYDWFARQKK		>10 μM			
RP-15		A6	SQAGSAFYAWFDQVLRTVKK					
RP-16		B6	VDARDDFUJLSESVTLL					
RP-17		B6	QSDAFYSGLWALGLSDGKK		>10 μM			
RP-18		B6	LQPCSGFYDYFWQRLHLGSKK					
RP-19		A6	LKQGFYDYFWQRLHLGSKK					
RP-20		B6	GSASFYDAIDRLLRMRIKK					
RP-24		GROUP 6	WPGYLFFEEALQDWRGSTED					
S167	S167	A6	AFYDWFAKK	>20 μM	No Binding			
S173	S173	RB6	LDALDRLMRYFEERPSL	1.2 μM				
S174	S174	RB6	PLAELWAYFEHSEQGRSSAH	16 μM				0
S175	S175	A6	GRYDWLQRNANFYDWFVAELG	230 nM	2-4 μM	0.9 nM	++	0
S176	S176	A6	NGVERAGTGDNFYDWFVAQLH	470 nM				+++

FIG. 11B

D Name	Clonal Name	Formula #	$K_4(\mu M)$ HIR	PO_4	Fat Cell Assay	Activity	$K_4(\mu M)$ HIGFR	Ratio IGF/IR	Sequence
D101	20D3	1	0.51 0.27				13 11	25 41	KIGGQGQHQDGNFYDWFVEALAKK (ε-biotin)
D102	20D1	1	1.2 0.97				7.4 16	6.2 16	KVLQARHGCDSVSDCFYEWFAKK (ε-biotin)
D103	B8	1	0.74				15	20	KWSALLSVMDTGFYAWFDDAVKK (ε-biotin)
D104	E7	1	20				>20	>1	KGHSWALVRHVDRLFYEWFDLKK (ε-biotin)
D105	H8	1	2.8				12	4.3	KRDKPTDQEEQNWSFYEWFRHKK (ε-biotin)
D106	20F1	1	0.97				6.2	6.4	KVFWNCRSQQLDFYEWWFEQAAKK (ε-biotin)
D107	40G11	1	1.1	YES		Antagonist	9.7	8.8	KLESHYWPQAALDRLFYSWFSKK (ε-biotin)
D108	3G11	1	2.3			Antagonist	19	8.3	KFYGWFSRQLSLTPRDDWGLPKK (ε-biotin)
D109	20H1	1	3.6			Antagonist	12	3.3	KSAPGLVSNKQDGLFYSWFREKK (ε-biotin)
D110	G3	1	0.84			Antagonist	1.4	1.7	KRGGGTFYEWFESALRKHGAGKK (ε-biotin)
D111	D2	1	0.62				3.2	5.2	KDPERMQSDVGFYEWFRAAVGKK (ε-biotin)
D112	IGFR C1 A65-4-C1	1	0.49 0.19			Neutral	0.05* 0.02*	0.1 0.1	DYKDCWARPCGDAANFYDWFVQQASKK (ε-biotin)
D113	IGFR H2 A65-4-1+2	1	0.75		-20 μ M	Agonist	5.4	7.2	DYKDVTFTSAVFHENFYDWFVRQVSKK (ε-biotin)
D114	IGFR A6	1	8.1			Neutral	>20	>2.5	SAKNFYDWFVKK (ε-biotin)
D115	IGFR D5	1	8.1				>20	>2.5	ADKNFYDWFMAAKK (ε-biotin)
D116	IGFR JBA5	9	4.4 cycli		>20 μ M	Agonist	8.1	1.8	DYKDLCQSWGVRIGWLAGLCPKK (ε-biotin)
D117	IGFR H2C	1	0.70	YES	-20 μ M	Agonist	6.1 5.1	8.6 8.5	FHENFYDWFVRQVSKK (ε-biotin)
D118	20E2	2	0.25	YES	-20 μ M	Antagonist	1.3	5.2	DYKDFYDAIDQLVRGSARAGGTRDKK (ε-biotin)
D119	20C11	2	0.25	YES	-20 μ M	Antagonist	13 2.5	2.9 0.8	KDRAFYNGLRDLVGAVYGAWDKK (ε-biotin)
D120	E8	10	0.37			Antagonist	2.2	5.9	KVRGFQGGTVWPGYEWLRNAAKK (ε-biotin)
D121	F2	10	1.1			Antagonist	7.4	6.7	KSMFVAGSDRWPGYGVLADWLKK (ε-biotin)
D122	20A4 (A7)	6	1.2 1.0			Antagonist	>20 >20	>17 >20	KEIEAEWGRVRCLVYGRCVGGKK (ε-biotin)
D123	D8	6	0.55 1.3			Antagonist	16 >20	29 >15	KWLDQEWAWVQCEVYGRGCPSKK (ε-biotin)
D124	F8	4	0.04* 0.09*				8.2 >20	200 >200	KHLCVLEELFWGASLFGYCSGKK (ε-biotin)
D125	IGFR E4	1	2.6				>20	>8	DYKDERSAAGFRGNFYDWFVAQVNKK (ε-biotin)
D126	IGFR D2C	1	1.4				18	13	LGENFYDWFVMQVRKK

FIG. 11A

Cl ne	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**HLSVLEELSWGASLFGQWAG**	**5.4**	**1.0**	**2.1**	**0.5**	**2.1**
NNKH-2-C5-IGFR	HL*VLEELSWGASLVGQWAV	7.3	0.9	0.7	1.3	0.8
NNKH-2-D9-IGFR	HLSVLEEL*LGASMFGLWAG	4.1	0.5	0.4	1.3	0.8
NNKH-2-H12-IGFR	HLSVLKELSW*ASLFGQWAG	5.0	1.3	1.1	1.2	0.8
NNKH-2-D10-IGFR	HLSALEELSWGASLFGQWAG	4.8	2.1	1.9	1.1	0.9
NNKH-2-G9-IGFR	HLSVLAELS*GALLFGQWAG	1.9	1.4	1.3	1.1	0.9
NNKH-2-C6-IGFR	RLSVLEQLSWGASLFGPWAG	18.2	1.0	0.9	1.1	0.9
NNKH-2-C7-IGFR	HL*VLVQPSWGASLFGQWAG	21.8	1.3	1.3	1.0	1.0
NNKH-2-F11-IGFR	HQSVLEELSR*ASLFGQWAG	6.7	1.3	1.4	0.9	1.1
NNKH-2-H3-IGFR	DMSVLGGLSWGA*LFGQWSG	4.7	0.7	0.8	0.9	1.1
NNKH-2-B8-IGFR	HLSVREGQLWRASMFGRWAG	17.5	3.7	5.2	0.7	1.4
NNKH-2-B12-IGFR	QLSVLVEL*WGASLFGPWAA	1.2	1.0	2.9	0.3	2.9
NNKH-2-F9-IGFR	HLSVGEELSW*VALLGQWAR	3.7	0.6	2.1	0.3	3.5

FIG. 10I

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**HLCVLEELFWGASLFGYCSG**	**39.1**	**1.8**	**27.7**	**0.1**	**15.4**
F815-4-G11-IGFR	HFYVLVERLSGASLFGSGSA	34.6	7.9	1.0	7.9	0.1
F815-3-D1-IGFR	HRFVREGLLWGAYQFCYCSG	14.9	1.0	2.0	0.5	2.0
F815-4-C12-IGFR	FQSLLEELVWGAPLFRYGTG	35.2	1.0	2.0	0.5	2.0
F815-4-A11-IGFR	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1

FIG. 10G

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/D sign	**HLSVLEELSWGASLFGQWAG**	**5.4**	**1.0**	**2.1**	**0.5**	**2.1**
NNKH-4-A9-IR	NLCRLEELAWGASLFGQCAG	16.3	1.0	2.7	0.4	2.6
NNKH-4-H4-IR	APVSTEELRWGALLFGQWAG	15.6	1.0	2.6	0.4	2.5
NNKH-4-B3-IR	HLSVLEERWWRESLFGQWAG	13.6	2.8	6.7	0.4	2.3
NNKH-4-E1-IR	HLSVLEERWWRAALFGQWAG	13.9	4.8	9.5	0.5	2.0
NNKH-4-E7-IR	HLSILEEQWWRESLFGQWAG	16.9	1.3	2.3	0.6	1.8
NNKH-4-G3-IR	HMSVEELSWWASLFGKQAG	11.3	1.3	2.3	0.6	1.7
NNKH-4-B6-IR	HLSELEERWWRATLFGQWAG	13.2	1.3	2.1	0.6	1.7
NNKH-4-A10-IR	HLSVLEELWWRESLFGQWAG	15.4	2.0	3.2	0.6	1.6
NNKH-4-A5-IR	HLSLLEEQWWRESLFGQWAG	14.6	4.6	6.9	0.7	1.5
NNKH-4-F11-IR	HLSVLEERWWRETLFGQWAG	14.0	3.1	3.9	0.8	1.3
NNKH-4-C9-IR	HLSVLEEQWWRESLFGQWAG	14.3	2.3	2.9	0.8	1.3
NNKH-4-D12-IR	HLSVLEEQWW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2
NNKH-4-D10-IR	HLSVLEELWWREALFGQWAG	13.6	1.2	1.5	0.8	1.2
NNKH-4-E5-IR	HLSVLEERWWRATLFGEWAG	14.5	1.4	1.6	0.9	1.1
NNKH-2-A6-IR	HL.VLEELLWGVSLFRQWAG	8.4	1.4	1.5	1.0	1.1
NNKH-4-F6-IR	HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0
NNKH-4-C7-IR	HLSVLEERWWRATLLESGQ	14.7	1.4	1.4	1.0	1.0
NNKH-4-F7-IR	HLSALEELWWRETLFGQWAG	14.1	7.5	7.0	1.1	0.9
NNKH-4-F8-IR	HLSVLEELWWRESLFGKWAG	13.6	11.0	8.6	1.3	0.8
NNKH-4-E9-IR	HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8
NNKH-4-E6-IR	HMSEQEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7
NNKH-4-B7-IR	HLSVLEERWWRETLFGEWAG	16.5	12.9	8.2	1.7	0.6
NNKH-2-B3-IR	HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1

FIG. 10H

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXCXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
20C-3-H3-IGFR	DHRLCGTDEYLMQDLFVRGLCRLIW	28.5	26.6	1.0	26.6	<0.1
20C-3-F4-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR	34.4	27.5	1.2	23.1	<0.1
20C-4-C10-IGFR	IWIACLDELLRGQVWSSCRRRAPIG	35.5	24.4	1.3	19.2	0.1
20C-3-G5-IGFR	DWLRCLGVILSGGLTELANTGCVQG	29.3	21.1	1.1	18.7	0.1
20C-3-A2-IGFR	WFSFCLGGLLQAQEWSVWGRDVGCI	33.9	18.3	1.1	16.9	0.1
20C-3-B4-IGFR	GYSWLRDVLMEKQAQLKREGSVGRQ	39.8	29.1	1.9	15.2	0.1
20C-3-C6-IGFR	FLTRLLERLGLS*ERGEAGGPYAQA	34.8	20.9	1.4	14.9	0.1
20C-3-E2-IGFR	FSGFCMGLERLSQVSLGYCGAGQGG	34.8	28.1	2.0	14.2	0.1
20C-3-A3-IGFR	ISFRCQLFVLAGMHPCPVDVGGEGF	33.7	14.3	1.2	12.4	0.1
20C-3-B1-IGFR	NTPNCSQDWGQESGFMALLLALTCK	30.2	9.8	0.9	11.2	0.1
20C-3-F5-IGFR	LQGFCELLATVTGVTGLGCLDYQPI	35.5	31.9	3.9	8.2	0.1
20C-4-A7-IGFR	GSSICNLLARAQIVELALCEMGVQE	33.3	19.3	2.8	6.9	0.1
20C-4-F8-IGFR	LSFACLLSQLSGVVLPDCLLGED	30.5	27.7	5.3	5.2	0.2
20C-4-G11-IGFR	GEHFCQLLMSLCGDDCGPVNCGGGS	24.7	13.3	2.8	4.7	0.2
20C-3-E1-IGFR	GWFECLLASLVLQVPQGRSRASAVC	34.0	5.1	1.6	3.1	0.3
20C-3-B6-IGFR	YRQECACSVGAVGFLCGLACLARSG	37.3	32.8	13.7	2.4	0.4

FIG. 10F-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAWPA.ARGRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
40F-3-A3-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	2.6	2.0	0.3	7.9	0.1
40F-4-C4-IGFR	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1	0.2

FIG. 10F-2

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-40A2-IR	RGTRTDRLWKSGGFAIVPRWPCFSYHCLVEWITKTGSPG	44.6	1.5	2.7	0.6	1.8
R40-4-40F10-IR	GRTSMAFVPPRHLQPELAPRPVRNHAWLVGGG	46.4	1.9	2.1	0.9	1.1

FIG. 10D

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20-4-F10-IGFR	CLGAGSFRAGILCLGGLPVS	35.5	6.0	--	--	--
R20-4-F7-IGFR	GFWATACGGLQICEELGLKP	29.1	4.7	--	--	--
R20-4-H9-IGFR	DLFCAYMAQALGLGQDLSCG	25.7	3.0	--	--	--
R20-3-A4-IGFR	RHLLLPQIWIAS*GGWGMG	15.6	2.7	--	--	--

FIG. 10E

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20β-4-A4-IR	WPGYLFFEEALQDWRGSTED	11.9	17.5	1.4	12.5	0.1
R20β-4-F2-IR	SMFVAGSDRWPGYGVLADWL	16.4	13.9	3.1	4.5	0.2
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1

FIG. 10B-1

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
20F-4-H10-IR	LDLASGDSWLGYDVLRGWLS	10.2	3.1	2.4	1.3	0.8
20F-4-C10-IR	IHSSDGIGAWGGYAWFRDVA	23.4	9.6	4.1	2.3	0.4

FIG. 10B-2

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20β-4-D10-IR	LGPLLRWGSEVCGVWPDLCE	21.5	1.0	8.0	0.1	8.0
R20β-4-D9b-IR	PFGFGGRWWGIPRMWWYRNS	32.6	6.8	15.1	0.5	2.2
R20β-4-H4-IR	WWWGGRNRWWLERWGLGGER	11.6	1.7	3.6	0.5	2.1
R20β-4-A2-IR	GRVALWGPVWPRWWFMSRPV	17.1	2.6	5.2	0.5	2.0

FIG. 10C

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20-4-C10-IGFR	PKGTRFRGDVDVWDGYSWLA	37.8	3.8	--	--	--

FIG. 10A-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
20F-4-B7-IGFR	TPIPAGGINIASWGGYTWLS	10.9	3.7	0.5	7.3	0.1
20F-4-E4-IGFR	HRGTVTGVWVARWPGYEWLS	8.9	4.7	0.7	6.3	0.2
20F-4-E12-IGFR	SDVWAQPQRRNDWPGYHWLS	9.7	4.7	0.8	6.0	0.2
20F-4-F4-IGFR	HRGTVTGVWVARWPGYEWLS	13.9	10.1	1.8	5.6	0.2
20F-4-F7-IGFR	SDVWAQPQRRNDWPGYHWLS	13.7	3.9	0.8	5.1	0.2
20F-4-E7-IGFR	RPHRINPQDDAVWPGYLWLG	7.2	2.5	0.5	4.7	0.2
20F-4-F11-IGFR	HRGTVTGVWVARWPGYEWLS	17.6	16.2	3.5	4.6	0.2
20F-4-D10-IGFR	FGRGYGGDGGGYWSGYEWLA	9.8	2.4	0.6	4.1	0.2
20F-4-B3-IGFR	DGLVVKSGREWPGYGWLER.A	17.3	14.4	3.6	4.0	0.2
20F-4-B12-IGFR	DGSIV.VSSSVGWPGYEWLM	10.1	9.9	2.4	4.0	0.2
20F-3-A9-IGFR	WQQANLSNGGGRWGGYDWLM	6.6	2.7	0.7	4.0	0.2
20F-4-G2-IGFR	FGRGYGGDGGGYWSGYEWLA	5.1	1.3	0.5	2.7	0.4
20F-4-D11-IGFR	VNYEMDRVPPMPWGGYWWLS	5.0	1.0	0.5	2.3	0.4
20F-4-G4-IGFR	MGGGLWVGVHIWPGYSWLSQ	3.9	0.9	0.5	1.8	0.6
20F-4-G12-IGFR	SDVWAQPQRRNDWPGYHWLS	3.2	0.9	0.6	1.5	0.7

FIG. 10A-2

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	LCQSWGVRIGWLAGLCP	--	--	--	--	--
JBA5-4-G12-IR	LCQSWDACIQWLVGLSP	37.5	3.0	1.4	2.1	0.5
JBA5-4-G3-IR	LCRSWEECIGWLVGPQP	4.5	2.5	1.1	2.3	0.4
JBA5-4-G1-IR	LCQSWGECIDRLVGQGA	32.0	3.2	1.3	2.5	0.4
JBA5-3-B1-IR	LCQGWGVRIGWLAGLCP	29.4	6.8	1.2	5.7	0.2
JBA5-3-C1-IR	LCQGWAVHIGQLAGLCP	36.3	7.5	1.1	6.8	0.1
JBA5-3-A6-IR	LCQGWGVHIGRLAGLCP	28.0	7.4	0.7	10.6	0.1
JBA5-3-A2-IR	LCQSWGVRIGWLAGLCP	10.2	4.8	0.4	12.0	0.1
JBA5-3-B7-IR	LCQSWGVHIGRLAGLCP	39.2	15.2	1.2	12.7	0.1

FIG. 9C

Clon	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
H5 Parental	**LCQSLGVTYPGWLAGWCA**	--	1.2	--	--	--
H5-3-JBA5-IGFR	LCQSWGVRIGWLAGLCP	31.9	16.3	--	--	--
H5-3-E1A11-IGFR	VCQSLGITDLGLCAGWGA	21.3	8.0	--	--	--
H5-3-E4B10-IGFR	LCQSLGLTHPGFEAWLCA	29.7	7.8	--	--	--
H5-3-E4C10-IGFR	LCQNFGVTDPGCFYGWFA	24.3	6.1	--	--	--
H5-3-JBB6-IGFR	PCQRLGDTHLCWLAGWFA	40.2	5.4	--	--	--
H5-3-E4A9-IGFR	LCQSSGLSFLGCLGWWA	27.7	4.3	--	--	--
H5-3-E2A12-IGFR	LCQSLGFTDLDWLACWFE	27.2	4.2	--	--	--
H5-3-E4A12-IGFR	VCQGLGVECPGWFAGWWA	27.9	3.9	--	--	--
H5-3-E1F9-IGFR	PCQSLGLTCSGWFEGWGA	18.6	3.5	--	--	--
H5-3-E4F11-IGFR	LCQGWGIRIGWLVGRCM	28.4	3.3	--	--	--
H5-3-E4A11-IGFR	LWQSVGIKYPGGLAGWLA	31.0	3.0	--	--	--
H5-3-E4G7-IGFR	QWQSLGVTCPGSWAELCA	26.2	2.2	--	--	--
H5-3-E1B9-IGFR	LCQSLGVTYWEGLAWLCA	20.0	2.1	--	--	--

FIG. 9A

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
JBA5 Parental	**LCQSWGVRIGWLAGLCP**	**31.5**	**20.6**	**1.0**	**20.6**	**<0.1**
JBA5-4-2C12-IGFR	LCQSWGVRIGWLAGLCP	46.8	41.5	1.0	41.5	<0.1
JBA5-2-1F9-IGFR	LCESWGVRIGWLAGLCP	48.1	39.5	1.0	39.5	<0.1
JBA5-2-1E10-IGFR	LCQSWGVRIGWLVGLCP	42.5	39.5	1.1	35.9	<0.1
JBA5-4-2A11-IGFR	LCQGWGVRIGWLAGLCP	44.1	40.2	1.2	33.5	<0.1
JBA5-3-2A3-IGFR	LCQSWGVRIGWLVGLCP	34.7	33.3	1.0	33.3	<0.1
JBA5-4-2A9-IGFR	LCQSWGVRIGWLTGLCP	34.6	33.1	1.0	33.1	<0.1
JBA5-1-1B6-IGFR	MCQSWDVRIGRLGGQCP	39.6	31.4	1.0	31.4	<0.1
JBA5-4-2B9-IGFR	LCQGWDVRIGQLAGLCP	39.6	22.3	1.0	22.3	<0.1
JBA5-1-1H7-IGFR	LCQGWGVRIGWLAGLCP	24.9	22.6	1.2	18.8	0.1
JBA5-3-2C3-IGFR	LCQSWDVRIGWVAGLCP	35.5	15.3	1.1	13.9	0.1
JBA5-1-1G7-IGFR	LCQSWDARIGWLAGLCP	26.2	14.8	1.5	9.9	0.1
JBA5-2-1E9-IGFR	LCLG*DVRIGLLAGLCP	39.4	4.5	1.0	4.5	0.2
JBA5-2-1D12-IGFR	L*KSWDVRSGLMAGLCP	42.2	2.2	1.0	2.2	0.5

FIG. 9B

J216	VCAWPTYWNCG	$2.8*10^{-6}$
J227	SFYEAIHQLLGV-NH_2	$6.4*10^{-6}$
J228	HPPLEHLKAFLL-NH_2	$2.4*10^{-5}$
J229	APTFYAWFNQQT-NH_2	$2.4*10^{-6}$
S122	HPTSKEIYAKLLK	$9.3*10^{-6}$
S123	HPSTNQMLMKLFK	$1.6*10^{-5}$
S124	HPPLSELKLFLIKK	$2.3*10^{-5}$

FIG. 8-4

J185	ACV D PTYWNCG	$3.4*10^{-5}$
J186	AC D WPTYWNCG	$3.5*10^{-5}$
J187	ACVWTYWNPCG	$4.3*10^{-5}$
J188	ACVWTYWPNCG	$3.0*10^{-5}$
J189	ACVWTYPWNCG	$3.1*10^{-5}$
J190	ACVWTPYWNCG	$2.6*10^{-5}$
J191	ACVPWTYWNCG	$3.0*10^{-5}$
J192	ACPVWTYWNCG	$4.2*10^{-5}$
J193	ACWPTYWNVCG	$4.8*10^{-5}$
J194	ACPTYWNVWCG	$4.2*10^{-5}$
J195	ACTYWNVWPCG	$3.3*10^{-5}$
J196	ACYWNVWPTCG	$2.4*10^{-5}$
J197	ACWNVWPTYCG	$2.9*10^{-5}$
J198	ACNVWPTYWCG	$4.2*10^{-5}$
J199	ACVWPCG	$4.7*10^{-5}$
J200	CVWPTYWNCG	$5.5*10^{-5}$
J201	ACWWPTYWNCG	$6.8*10^{-6}$
J202	ACEWPTYWNCG	$4.6*10^{-6}$
J203	ACRWPTYWNCG	$5.8*10^{-6}$
J204	ACQWPTYWNCG	$9.2*10^{-6}$
J205	ACGWPTYWNCG	$4.4*10^{-6}$
J207	cyclo-Valeroyl-AWPTYWNCG	$5.5*10^{-5}$
J208	cyclo-Toluyl- AWPTYWNCG	$7.6*10^{-5}$
J209	cyclo-Acetyl- AWPTYWNCG	$7.7*10^{-5}$
J210	$(WPTYWNCG)_2$	$5.3*10^{-5}$
J211	$(AWPTYWNCG)_2$	$7.9*10^{-6}$
J212	ACA(Bpa)PTYWNCGK(biotin)	$1.8*10^{-5}$
J213	ACAWPTY(Bpa)NCGK(biotin)	$1.8*10^{-5}$
J214	GCAWPTYWNCG	$1.4*10^{-6}$
J215	NCAWPTYWNCG	$9.0*10^{-6}$

FIG. 8-3

J137	AC(dMet)WPTYWNCG	$2.5*10^{-5}$
J138	AC(Abu)WPTYWNCG	$7.8*10^{-5}$
J139	AC(dAbu)WPTYWNCG	$2.1*10^{-5}$
J140	AC(Nva)WPTYWNCG	$3.6*10^{-6}$
J141	AC(dNva)WPTYWNCG	$3.0*10^{-5}$
J142	AC(tBuG)WPTYWNCG	$3.2*10^{-5}$
J143	AC(dtBuG)WPTYWNCG	$3.8*10^{-5}$
J144	AC(Phe)WPTYWNCG	$5.1*10^{-6}$
J145	AC(dPhe)WPTYWNCG	$5.7*10^{-5}$
J146	AC(Cha)WPTYWNCG	$2.2*10^{-5}$
J147	AC(dCha)WPTYWNCG	$1.7*10^{-5}$
J148	AC(Nal(1))WPTYWNCG	$5.8*10^{-6}$
J149	AC(dNal(1))WPTYWNCG	$2.0*10^{-5}$
J150	AC(Acy)WPTYWNCG	$2.0*10^{-5}$
J151	ACVWPT(Hyp)WNCG	$2.2*10^{-4}$
J154	ACVWPT(Nal2)WNCG	$8.2*10^{-5}$
J155	ACVWPT($MetO_2$)WNCG	$1.9*10^{-4}$
J157	ACVWPT(Cha)WNCG	$1.2*10^{-4}$
J160	ACVWPT(Ser)WNCG	$1.8*10^{-4}$
J162	ACVWPT(Thi)WNCG	$2.5*10^{-4}$
J163	ACVWPT(dSer)WNCG	$5.0*10^{-5}$
J166	ACVWPT(dCha)WNCG	$7.5*10^{-5}$
J170	ACVWPT(dPhe)WNCG	$1.4*10^{-4}$
J171	ACVWPT(Thr)WNCG	$7.7*10^{-4}$
J174	ACVWPT(Phe)WNCG	$4.5*10^{-5}$
J176	ACVWPT(dThr)WNCG	$2.8*10^{-5}$
J180	ACVWPTYW D CG	$5.6*10^{-5}$
J182	ACVWPT D WNCG	$2.7*10^{-5}$
J183	ACVWP D YWNCG	$3.3*10^{-5}$
J184	ACVW D TYWNCG	$6.2*10^{-5}$

FIG. 8-2

J-nr	Sequence	HIR affinity mol/l
J101	ACVWPTYWNCG	$5.0*10^{-6}$
J103	Ac-CVWPTYWNCG	$3.0*10^{-5}$
J104	Bz-CVWPTYWNCG	$3.2*10^{-5}$
J105	Ac-ACVWPTYWNCG	$4.5*10^{-5}$
J109	ACVWPTYWACG	$2.0*10^{-5}$
J110	ACVWPTYANCG	$2.4*10^{-5}$
J111	ACVWPTAWNCG	$3.1*10^{-5}$
J112	ACVWPAYWNCG	$3.3*10^{-5}$
J113	ACVWATYWNCG	$5.5*10^{-5}$
J115	ACAWPTYWNCG	$2.7*10^{-6}$
J116	AAVWPTYWNAG	$3.4*10^{-5}$
J117	ASVWPTYWNSG	$2.9*10^{-5}$
J118	ACPYNWVTWCG	$2.9*10^{-5}$
J119	ACVWPTYWnCG	$3.2*10^{-5}$
J120	ACVWPTYwNCG	$3.4*10^{-5}$
J121	ACVWPTyWNCG	$1.8*10^{-5}$
J122	ACVWPtYWNCG	$5.1*10^{-5}$
J123	ACVWpTYWNCG	$2.5*10^{-5}$
J124	ACVwPTYWNCG	$2.0*10^{-5}$
J125	ACvWPTYWNCG	$1.8*10^{-5}$
J127	acvwptywncg	$4.4*10^{-5}$
J128	gcnwytpwvca	$5.3*10^{-5}$
J130	AEVWPTYWN(Dpr)G	$1.9*10^{-5}$
J131	ACDWPTYWNCG	$5.5*10^{-5}$
J132	AC(Leu)WPTYWNCG	$4.5*10^{-6}$
J133	AC(dLeu)WPTYWNCG	$2.8*10^{-5}$
J134	AC(Ile)WPTYWNCG	$7.4*10^{-6}$
J135	AC(dIle)WPTYWNCG	$2.9*10^{-5}$
J136	AC(Met)WPTYWNCG	$7.5*10^{-6}$

FIG. 8-1

	Sequence	HIR affinity mol/l
J228	HPPLEHLKAFLL-NH_2	$2.4*10^{-5}$
J229	APTFYAWFNQQT-NH_2	$2.4*10^{-6}$
S122	HPTSKEIYAKLLK	$9.3*10^{-6}$
S123	HPSTNQMLMKLFK	$1.6*10^{-5}$
S124	HPPLSELKLFLIKK	$2.3*10^{-5}$

FIG. 7

Clon	Sequence	Ratios over Background			Comparisons	
Parental/Design	WLDQEWAWVQCEVYGRGCPS	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		44.8	1.4	24.2	0.1	17.3
D820-4-G9-IGFR	WLDQEWAQVQCEVWGRGCPS	36.8	1.0	29.6	<0.1	29.6
D820-4-C10-IGFR	WLDLEWEFVQCEVYGRGCPT	32.6	1.0	31.3	<0.1	31.3
D820-4-A9-IGFR	WLEQEWASVQCEVYGRGCPS	20.4	1.0	31.4	<0.1	31.4
D820-4-B8-IGFR	WLDLEWEQIKCKVYGRGCPF	31.1	1.0	32.7	<0.1	32.7
D820-4-F8-IGFR	WLEQEWAQIQCQIYGRGCPS	28.3	1.0	32.9	<0.1	32.9
D820-4-H7-IGFR	WLEQEWALVLCEVYGHGCPA	34.1	1.0	32.9	<0.1	32.9
D820-4-E8-IGFR	WLEQEWAQIQCEVWGRGCSS	26.6	1.0	33.2	<0.1	33.2
D820-4-G10-IGFR	WLE?EWEWVQCEVYGRGC?S	37.5	1.0	33.2	<0.1	33.2
D820-4-D10-IGFR	WLEQEWAQVQCDVYGRGCPS	36.6	1.0	33.5	<0.1	33.5
D820-4-D8-IGFR	WLEQE*ARVQCEVWGRGCPS	23.7	1.0	34.6	<0.1	34.6
D820-4-A10-IGFR	WL?QEWARVHCEVWGRP?QC	29.4	1.0	35.5	<0.1	35.5
D820-4-B7-IGFR	PLEHEWAWVQCVVYGRGCRS	35.4	1.0	36.9	<0.1	36.9
D820-4-E12-IGFR	SLE?EWAWVQCEV?GRGCP?	37.0	1.0	37.0	<0.1	37.0
D820-4-H10-IGFR	WLDQEWVRVQCEVWGRGCPS	36.8	1.0	37.1	<0.1	37.1
D820-4-F12-IGFR	SLDKEWAWVKCEVYGRGCPS	36.9	1.0	37.3	<0.1	37.3
D820-4-F7-IGFR	LGDQEWAWVEWEV#GRGWPS	34.4	1.0	37.5	<0.1	37.5
D820-4-G12-IGFR	WLEEEWAQIRCGVYGRGCPS	30.3	1.0	37.8	<0.1	37.8
D820-4-D12-IGFR	WLEEE*GWVQCEVWGRGCPP	37.2	1.0	38.6	<0.1	38.6
D820-4-A12-IGFR	CLDQEWA?VQCPVYGRGCPS	30.4	1.0	39.3	<0.1	39.3
D820-4-C12-IGFR	QLELEWARVQCEVWDRGCPS	37.1	1.0	39.6	<0.1	39.6
D820-4-A7-IGFR	RLEQEWAWIQCEVYGRGCRF	35.4	1.0	40.8	<0.1	40.8
D820-4-B12-IGFR	SLEHE*AWVQCKVYGRGC?S	36.2	1.0	41.4	<0.1	41.4

FIG. 6D-2

Cl ne	Sequence	Ratios over Background			Comparisons	
Parental/D sign	WLDQEWAWVQCEVYGRGCPS	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		44.8	1.4	24.2	<0.1	17.3
B6-4-G12-IR	WLDQEWAWIQCEVYGRGCPP	4.4	1.0	6.9	0.1	7.1
B6-3-A11-IR	WLDQEWAQVRCEVYGRGCPS	7.3	1.0	6.3	0.2	6.3

FIG. 6E

Clone Parental/Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	WLDQEWAWVQCEVYGRGCPS	**44.8**	**1.4**	**24.2**	**0.1**	**17.3**
D820-3-D5-IGFR	WVNQALGGVQSDVQGRRCQS	29.6	3.8	1.0	3.8	0.3
D820-3-E4-IGFR	LLDHEWPWVGCEVCGRGSLS	27.1	3.2	1.0	3.2	0.3
D820-3-C5-IGFR	WLHQELAWVRGEGYPRGRRS	25.0	3.1	1.0	3.1	0.3
D820-3-F4-IGFR	WLGHDWAWIQCEVYGLGCPC	3.9	2.7	1.0	2.7	0.4
D820-3-F6-IGFR	WIDQEGVRVQCEA*GRAFPS	26.7	2.6	1.0	2.6	0.4
D820-3-G4-IGFR	WRDEEWAWVQGVVQGRGWPA	3.8	2.6	1.0	2.6	0.4
D820-3-E2-IGFR	RLGVEWSWFQRKVYGRDSTS	15.3	2.6	1.0	2.6	0.4
D820-3-G6-IGFR	WLAQGWAGVQCVVYGRGCRN	20.3	2.4	1.0	2.4	0.4
D820-4-E11-IGFR	WLEEE*AGIQCQV?GRGCPS	12.6	1.0	3.0	0.3	3.0
D820-4-H11-IGFR	WLDQEWEWVQCEVWGRGCLS	8.1	1.0	4.6	0.2	4.6
D820-4-D11-IGFR	RLEQEWALIQCEVYGRGCPS	4.5	1.0	5.3	0.2	5.3
D820-4-A8-IGFR	WLEEEWAQVQCQVYGRGCAS	3.2	1.0	5.5	0.2	5.5
D820-4-F9-IGFR	WLDLE*EWLQCEVYGRGCAT	9.4	1.0	5.8	0.2	5.8
D820-4-C8-IGFR	WLEQEWVQVRCEVYGRGCPS	11.6	1.0	5.9	0.2	5.9
D820-4-D9-IGFR	WLEEEWAQVQCEVYGRGCPS	10.1	1.0	8.9	0.1	8.9
D820-4-D7-IGFR	WLDQEWARVQCEVWGRGCTY	34.1	3.5	33.4	0.1	9.5
D820-4-H9-IGFR	YLD?EWAWVQCEVYGLGCQS	18.4	1.0	10.1	0.1	10.1
D820-4-E10-IGFR	WLDVE*AWVQCEVWGRGCPS	26.7	2.6	27.0	0.1	10.4
D820-4-E7-IGFR	WLEQEWER?QCEVYGRGCPP	31.9	3.0	32.2	0.1	10.7
D820-4-H8-IGFR	WLEEEWAQVQCEVYGRGCLS	16.1	1.0	11.7	0.1	11.7
D820-4-A11-IGFR	WLDQEWAWIQCEVYGRGCPS	8.0	1.0	12.5	0.1	12.5
D820-4-C9-IGFR	?LEHEWAQIQCEV?GRGCQS	19.6	1.0	14.9	0.1	14.9
D820-4-E9-IGFR	WL?QEWAWIQCEVYGRGCPF	19.3	1.0	17.3	0.1	17.3
D820-4-B10-IGFR	WLD?EWAWVQCEVYGRGCPS	19.3	1.0	21.5	<0.1	21.5
D820-4-F10-IGFR	GLEQGCPWVGLEVQCRGCPS	27.8	1.0	25.7	<0.1	25.7
D820-4-B9-IGFR	WLEEEWAWVQCEVYGHGCPS	31.7	1.0	26.5	<0.1	26.5
D820-4-G8-IGFR	WLDQEWAQIQCEVYGRGCSS	25.6	1.0	29.3	<0.1	29.3

FIG. 6D-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	**44.8**	**1.4**	**24.2**	**0.1**	**17.2**
D820-3-E5-IR	WLEQEWTWVQCEVYGCGCPS	25.9	1.0	22.6	<0.1	22.6
D820-3-D1-IR	WLEKEWAGVQCEIYGRGCPS	27.3	1.0	22.4	<0.1	22.4
D820-3-E1-IR	WLEEEWAWVRCEVYGRGCQS	22.4	1.0	21.9	<0.1	21.9
D820-3-F1-IR	WLEHEWAQIQCELYGRGCTY	22.0	1.0	21.0	<0.1	21.0
D820-3-B2-IR	ALEEEWAWVQCEVYGRGCPS	13.1	1.0	18.4	0.1	18.4
D820-3-A3-IR	WLEQEWAQVQCEVYGRGCPS	23.5	1.0	18.4	0.1	18.4
D820-3-H4-IR	WLDDEWAQIQCEIYGRGCQS	25.6	1.0	17.5	0.1	17.5
D820-3-G1-IR	QLEEEWAGVQCEVYGRECPS	14.5	1.0	16.3	0.1	16.3
D820-3-C1-IR	WLEQEWLLVQCGVYGRGCPS	27.8	1.0	13.9	0.1	13.9
D820-3-A1-IR	WLDQEWAWIQCEVYGRGCRS	14.7	1.0	12.8	0.1	12.8
D820-3-A5-IR	WLEQEWAQVQCEVSGRGCPS	6.4	1.0	6.3	0.2	6.3
D820-3-H1-IR	W?DQEWALIQCEVYGRGCPS	13.7	1.0	6.2	0.2	6.2
D820-3-A4-IR	SLDEEWAGVLCEVYGRGCPF	6.0	1.0	4.3	0.2	4.3
D820-4-E12-IR	SVDQELEWLMCHFQGRVCPS	34.9	9.0	10.9	0.8	1.2
D820-4-B12-IR	WLEQERAWIWCEIQGSGCRA	32.2	8.6	1.0	8.6	0.1

FIG. 6C-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.2
D820-3-H2-IR	RLDLEWANIQCEVYGRGCPS	23.9	1.0	40.0	<0.1	40.0
D820-3-C4-IR	WLEQEWARVQCEVYGRGCSS	31.0	1.0	39.5	<0.1	39.5
D820-3-C3-IR	WLEQEWILVECEVYGRGCPT	35.2	1.0	39.4	<0.1	39.4
D820-3-G6-IR	WLEQEWAQVQCEVWGRGCPS	33.8	1.0	38.8	<0.1	38.8
D820-3-D2-IR	WLDQEWEWIQCEVYGRGCPL	35.6	1.0	37.8	<0.1	37.8
D820-3-D3-IR	LLDEEWAQIECEIYGRGCPS	34.8	1.0	37.7	<0.1	37.7
D820-3-B5-IR	ALEEEWAWVQCEVYGRGCHF	34.1	1.0	37.1	<0.1	37.1
D820-3-E2-IR	C?EQEWGLVQCEVYGRGCPS	34.4	1.0	37.0	<0.1	37.0
D820-3-B3-IR	WLEQEWAYVQCEVYGRGCPS	33.6	1.0	36.7	<0.1	36.7
D820-3-B6-IR	WLEHEWAQVQCEVWGRGCPY	31.2	1.0	36.6	<0.1	36.6
D820-3-D4-IR	WLEQEWAEVRCEVYGRGCPR	32.0	1.0	36.2	<0.1	36.2
D820-3-C2-IR	?LEQEWAWVQCEVYGRGCPS	33.7	1.0	35.6	<0.1	35.6
D820-3-F6-IR	WLEQEWAGIQCKVYGRGCPS	30.8	1.0	35.2	<0.1	35.2
D820-3-D5-IR	RLEQEWAQVQCEVWGRGCLP	30.5	1.0	34.8	<0.1	34.8
D820-3-F5-IR	QLDHEWAGIQCEVWGRGCPS	29.8	1.0	34.6	<0.1	34.6
D820-3-H3-IR	WLEQEWAQIQCEVYGAGCRS	30.2	1.0	33.8	<0.1	33.8
D820-3-G2-IR	SLEQEWAWVQCVVYGRGCPI	31.3	1.0	33.0	<0.1	33.0
D820-3-H6-IR	WLEQEWDQVLCEVYGRGCPY	30.3	1.0	32.2	<0.1	32.2
D820-3-F3-IR	WLEQEWAQV?CEVYGRGCA?	28.6	1.0	30.7	<0.1	30.7
D820-3-B4-IR	WMDQEWAWVQCEVYGRGCPS	33.1	1.0	30.5	<0.1	30.5
D820-3-C5-IR	QLDQEWAWIQCEVYGRNCRT	29.1	1.0	30.3	<0.1	30.3
D820-3-F4-IR	TLEQEWAQVICEVYGRGCLS	25.9	1.0	29.5	<0.1	29.5
D820-3-H5-IR	RLEQEWAQVQCEVWGRGCLS	26.3	1.0	28.6	<0.1	28.6
D820-3-A6-IR	WLDQEWALVQCEVYGRGCPA	24.8	1.0	26.0	<0.1	26.0
D820-3-A2-IR	WLDQEWAQIQCHVWGRGCPA	23.7	1.0	25.6	<0.1	25.6
D820-3-G5-IR	WLEQEWAWVQCEVYGRGCPS	22.6	1.0	25.0	<0.1	25.0
D820-3-G3-IR	RLEEEWAWVQCQVYGRGCPS	22.2	1.0	23.9	<0.1	23.9
D820-3-E3-IR	WLEQEWVRIQCEVYGRGCPS	20.6	1.0	22.7	<0.1	22.7

FIG. 6C-1

Clone	Sequence	Ratios over Background			Comparisons	
Parental/Design		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	WLDQEWAWVQCEVYGRGCPS	--	--	--	--	--
D815-4-G8-IR	QLDQEWARVRCEVWGRGCSS	27.8	1.0	33.6	<0.1	33.6
D815-4-G7-IR	WLDLEWAQVQCKVYGRGCPS	34.7	1.0	32.3	<0.1	32.3
D815-4-G11-IR	WLDEEWAWVQCQVYGRGCPS	30.7	1.0	28.6	<0.1	28.6
D815-4-E7-IR	WLDQEWAWVQCEVWGRGCAF	33.0	1.0	26.4	<0.1	26.4
D815-4-A12-IR	WLDREWAQVQCEVYGRGCLS	28.4	1.0	19.0	0.1	19.0
D815-4-B11-IR	WLDAEWEWVQCEVYGRGCRP	22.1	1.0	18.8	0.1	18.8
D815-4-D8-IR	SLDREWAYVQCQVYGRGCSS	20.8	1.0	14.6	0.1	14.6

FIG. 6B-2

Clone	Sequence	Ratios over Background			Comparis ns	
Parental/Design	WLDQEWAWVQCEVYGRGCPS	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		44.8	1.4	24.2	<0.1	17.3
D815-4-A8-IR	WLDLEWAQVQCEVYGRGCPS	48.0	1.0	48.4	<0.1	48.4
D815-4-D10-IR	WLDQEWAQVQCEVFGRGCPS	49.2	1.0	48.2	<0.1	48.2
D815-4-D9-IR	WLDQEWQQVQCQVYGRGCTS	47.5	1.0	48.0	<0.1	48.0
D815-4-A11-IR	RLDEEWARVQCEVWGRGCRS	47.9	1.0	48.0	<0.1	48.0
D815-4-E12-IR	WLEQEWAWIQCEVYGRGCPS	49.0	1.0	47.6	<0.1	47.6
D815-4-B7-IR	WLEQEWAQVQCEVYGRGCPS	45.4	1.0	47.2	<0.1	47.2
D815-4-D11-IR	WLDEEWEWIQCKVYGRGCPA	49.5	1.0	47.0	<0.1	47.0
D815-4-D12-IR	WLEQEWAWVQCEVYGRGCQS	48.1	1.0	46.6	<0.1	46.6
D815-4-F8-IR	WLDQEWAWIQCEVYGRGCPA	47.8	1.0	46.4	<0.1	46.4
D815-4-A9-IR	SLDWEWAWLQCEVYGRGCPS	47.7	1.0	45.8	<0.1	45.8
D815-4-E9-IR	WLEQEWEQVRCLVYGRGCPP	47.8	1.0	45.8	<0.1	45.8
D815-4-B10-IR	WLDQEWAWVQCEVYGRGCPY	49.0	1.0	45.6	<0.1	45.6
D815-4-H8-IR	WLDQEWAGVLCEVYGRGCPS	49.0	1.0	45.6	<0.1	45.6
D815-4-E10-IR	SLDKEWEWVLCVVYGRGCPS	47.0	1.0	45.6	<0.1	45.6
D815-4-D7-IR	WLEQEWAQVQCEVYGRGCRS	44.5	1.0	45.4	<0.1	45.4
D815-4-G9-IR	WLEEEWAQVQCAVYGRGCSS	44.2	1.0	44.2	<0.1	44.2
D815-4-G12-IR	WLDQEWALVQCEVYGRGCPS	44.3	1.0	43.7	<0.1	43.7
D815-4-E11-IR	WLDQEWAWVQCEVYGRGCPS	45.5	1.0	43.0	<0.1	43.0
D815-4-H7-IR	WLEQEWAWVQCEVYGRGCAS	46.2	1.0	43.0	<0.1	43.0
D815-4-F12-IR	WLDQEWAWVECEVYGRRCPS	47.2	1.0	42.6	<0.1	42.6
D815-4-E8-IR	WLDQEWAWVECQVYGRGCPS	47.9	1.0	42.6	<0.1	42.6
D815-4-F9-IR	QLDQEWAWVLCKVYGRGCPS	46.4	1.0	41.8	<0.1	41.8
D815-4-A10-IR	WLDHE*AWVQCEVYGRGCPS	47.3	1.0	41.2	<0.1	41.2
D815-4-C7-IR	QLEQEWAWVRCEVYGRGCSS	37.7	1.0	40.0	<0.1	40.0
D815-4-H10-IR	WLDQEWAWVQCQVYGRGCLS	47.0	1.0	39.8	<0.1	39.8
D815-4-C9-IR	WLDQEWAWVRCEVYGLGCPS	44.2	1.0	39.8	<0.1	39.8
D815-4-F11-IR	WLDQEWAVMKCELYGRGCPS	40.4	1.0	39.2	<0.1	39.2
D815-4-H12-IR	WLEQEWAWVQCEVYGRGCLS	45.4	1.0	38.6	<0.1	38.6
D815-4-A7-IR	SLDQEWAWVQCEVYGRGCLS	37.3	1.0	37.3	<0.1	37.3
D815-4-H11-IR	WLDHEWAWVQCEVYGRGCTS	2.4	1.0	37.2	<0.1	37.2
D815-4-F7-IR	WLDVEWAWVQCEVYGRGCPS	32.4	1.0	34.7	<0.1	34.7

FIG. 6B-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	xxxxxxxxxxxxxxxxxxxx	--	--	--	--	--
R20α-3-20A4-IR	EIEAEWGRVRCLVYGRCVGG	50.2	1.6	23.1	0.1	14.4
R20β-4-A7-IR	EIEAEWGRVRCLVYGRCVGG	44.2	1.3	24.0	0.1	18.5
R20β-4-D8-IR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3

FIG. 6A

Clon Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20C-3-A1-IR	RLCALEELFWGASLFGQCSG	21.0	1.1	17.6	0.1	16.6
20C-3-C1-IR	HLCVLEELFWGAALFHQCSG	30.6	1.4	21.9	0.1	16.1
A6L-3-D2-IR	RLCVLEEQFWGASLFGQCSG	7.0	1.1	14.9	0.1	14.1
B6C-4-G12-IR	QLCVLEELFWGSSRLGYCSG	31.1	2.5	33.5	0.1	13.6
B6H-4-F9-IR	DLCVLEELFWGASLFGQCSG	39.3	3.6	43.1	0.1	12.1
B6C-4-E3-IR	QLCLLEEQFWGGSLFGQCSG	34.6	5.3	40.0	0.1	7.6
20C-3-B10-IR	HLCVLEELFWGTSLFGQCSG	29.9	16.9	31.7	0.5	1.9
20C-3-A3-IR	RLCVLEELVWGASLFDQCSR	28.4	19.1	25.3	0.8	1.3

FIG. 4E-3

Clone Parental/Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
F815-4-D10-IGFR	**PLQALCEKFFGAWMFGYCSG**	**39.1**	**1.8**	**27.7**	**0.1**	**15.4**
F815-4-H11-IGFR	HLQVLCELFGGVYLFGYCSG	31.2	13.9	1.0	13.9	0.1
F815-4-C8-IGFR	PLFDLCELFGGASLSGYCYG	27.2	19.2	1.7	11.3	0.1
F815-4-E8-IGFR	HL*ALCELFGGVWSFGYCVG	35.4	17.4	1.6	10.9	0.1
F815-4-E11-IGFR	QLGVLCEMFGGAFRLGYCQG	29.5	16.9	1.7	9.9	0.1
F815-4-A7-IGFR	HLQDLCELFGGAYLFGYCSG	36.6	25.7	2.7	9.5	0.1
F815-3-D3-IGFR	QLQVLCELFGGAVSLRLLLW	29.6	16.0	3.8	4.2	0.2
F815-4-F7-IGFR	PLGVLCEQFGGAFRFGYCSG	33.7	3.5	1.0	3.5	0.3
F815-4-A9-IGFR	PL*GLCELFGGASLFGYCSS	33.6	18.9	9.9	1.9	0.5
F815-4-B12-IGFR	DLRVLCELFGGAYVLGYCSE	7.5	1.7	2.3	0.7	1.4
		35.1	3.7	12.6	0.3	3.4

FIG. 5

Clone Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20C-3-B4-IR	NLCVLEELFWGESLFGQCSG	28.9	1.1	31.1	<0.1	28.0
20C-3-C11-IR	HLCVLEEQFWGGSLFGYCSR	30.2	1.1	31.0	<0.1	27.7
B6C-4-G2-IR	HLCFLEEVFWGAALFAQCSG	29.4	1.3	35.3	<0.1	27.5
20C-3-B8-IR	HLCDLEVLFWGSALFGQCSG	28.5	1.1	31.2	<0.1	27.4
20C-3-C10-IR	HLCVMEELFWGASLFGQCSG	32.1	1.2	33.6	<0.1	27.1
20C-3-B6-IR	HLCVLEERFWGASLFWQCSG	29.7	1.2	31.9	<0.1	26.7
A6L-3-A3-IR	HLCVLEEQYWGESLFGYCSG	14.4	1.1	28.3	<0.1	26.5
A6L-3-B3-IR	PLCVLEEQFWGASLFAYCSS	38.7	1.7	43.4	<0.1	26.3
20C-3-A5-IR	QLCVLEELFWGESLFAQCLG	22.9	1.1	27.6	<0.1	26.0
20C-3-B11-IR	HLCVLEELFWGQSLFGHCSD	30.0	1.3	32.7	<0.1	25.8
20C-3-B3-IR	HLCVLEELVWGASLFGFCSG	29.3	1.2	31.2	<0.1	25.7
20C-3-C12-IR	LLCVLEEQFWGASLFGQCSG	29.6	1.3	31.8	<0.1	24.8
20C-3-C3-IR	RLCVLEELFWGESLFGQCSG	30.1	1.2	30.1	<0.1	24.3
20C-3-C2-IR	HLCVLEEMFWGASLFGNCSG	29.9	1.3	29.8	<0.1	23.8
20C-3-A11-IR	ELCFLEELFWGASLFGQCSG	25.9	1.2	27.4	<0.1	23.0
20C-3-A4-IR	HLCVLEELFWGASLYGQCSS	27.2	1.2	27.5	<0.1	22.9
20C-3-A6-IR	HLCVLEELFWGASLFAQCPG	26.1	1.2	27.5	<0.1	22.8
B6C-4-E4-IR	NLCVLEELFWGASEFGQCSG	34.5	1.7	39.1	<0.1	22.7
20C-3-A9-IR	DLCVLEEQLWGASLFRYCSG	29.7	1.3	29.3	<0.1	22.7
B6C-3-C5-IR	HLCVLEEQFWGVALFGNCSG	33.5	1.7	37.7	<0.1	22.5
20C-3-B1-IR	HLCVLEVQIWGASLFGQCSG	30.2	1.2	26.7	<0.1	22.0
20C-3-A10-IR	HLCVLEERFWGGALFGQCTA	29.0	1.3	28.5	<0.1	21.5
20C-4-F1-IR	HLCDLEELFWGASLFGQCSG	29.1	1.4	29.5	<0.1	20.7
20C-4-E1-IR	QLCVLEELFWGTSLFAGCSG	28.3	1.4	29.7	<0.1	20.6
20C-3-B12-IR	QLCGLEELFWGASLFGYCSA	27.0	1.3	25.8	<0.1	20.2
20C-3-A8-IR	HLCVLEELFWGASLFGQCSS	21.1	1.1	21.2	0.1	20.0
20C-3-A7-IR	FLCVLEELYWGASQFGQCSG	21.9	1.3	23.0	0.1	18.3
B6C-4-E10-IR	HLCVLEEQFWGASLFGYCSG	35.2	2.2	38.0	0.1	17.5

FIG. 4E-2

Clone	Sequence	Ratios over Background			Comparisons	
Design	HLCVLEELFWGASLFGYCSG	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
A6L-3-C4-IR	DLCVLEERFWGASLFGQCSG	36.9	1.0	40.5	<0.1	42.5
A6L-3-D7-IR	QLCVLEELHWGASLFGYCSG	38.6	1.0	40.1	<0.1	40.7
A6L-3-A1-IR	PLCVLEEQFWGASLFGQCSG	39.6	1.1	44.8	<0.1	40.6
A6L-3-C1-IR	YLCDLEERFWGASLFGQCSS	37.3	1.0	40.3	<0.1	40.3
A6L-3-D5-IR	HLCLLEERFWGSSQFGFCSG	42.9	1.1	44.4	<0.1	40.2
A6L-3-A4-IR	HLCVLEELFWGASQFGQCSG	26.7	1.1	42.2	<0.1	40.2
A6L-3-D3-IR	HLCYLEERFWGASLFGQCSG	34.6	0.9	36.9	<0.1	39.8
A6L-3-B1-IR	HLCVMEELFWGTSLFGQCTG	33.9	1.0	38.7	<0.1	39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSG	35.3	1.1	42.4	<0.1	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFSQCSG	38.1	1.1	42.7	<0.1	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSG	31.6	1.1	39.6	<0.1	36.7
B6C-4-H10-IR	QLCLLEELFWGAASFGQCSG	38.5	1.1	41.1	<0.1	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSG	31.7	1.1	39.7	<0.1	36.2
A6L-3-D6-IR	HLCDLEELFWGASLFSQCSR	35.5	1.0	37.2	<0.1	36.1
B6C-4-F1-IR	QLCVLEELFWGASQFGYCSG	32.9	1.1	38.7	<0.1	35.8
B6C-4-H3-IR	QLCALEEQFWGASLFSQCSG	37.4	1.2	40.5	<0.1	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSG	30.2	1.0	35.7	<0.1	34.3
B6C-4-G1-IR	HLCVLEEWFWGDSLFGQCSR	34.9	1.2	40.2	<0.1	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSG	34.4	1.2	38.8	<0.1	33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSG	34.7	1.2	39.6	<0.1	32.8
B6C-4-F11-IR	HLCVLEELFWGASRFGQCSG	34.0	1.2	37.2	<0.1	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSA	32.3	1.2	37.4	<0.1	30.6
B6C-4-E12-IR	HLCVLEELIWGASRFGQCSG	30.9	1.1	33.3	<0.1	30.2
B6C-4-G10-IR	HLCVLEELFWGGSLFIQCSG	33.0	1.3	40.3	<0.1	30.1
B6C-4-F8-IR	QLCVLEEQFWGASLFGNCSG	36.4	1.4	39.8	<0.1	29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSG	26.6	1.1	32.5	<0.1	29.2
B6C-4-G3-IR	HLCILEEMFWGASLFGQCGG	34.0	1.4	38.8	<0.1	28.3
20C-3-B7-IR	PLCVLEELVWGASLFVQCSG	29.5	1.2	32.9	<0.1	28.3

FIG. 4E-1

Clone Parental/Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
F820-4-A8-IR	QLCVMEELFWGASRFGQCSG	6.4	0.7	2.4	0.3	3.4
F820-4-G1-IR	HLCVLEELFWGASMFGQCSG	3.9	0.6	1.9	0.3	3.4
F820-4-F3-IR	QLCVLEEMFWGGSRFVQCSA	9.8	1.3	3.6	0.4	2.9
F820-4-D6-IR	PLCILEELFWGEALFDQCGA	5.4	1.2	3.2	0.4	2.6
F820-4-A1-IR	YLCVQEELFWGASLFGYCSV	25.5	2.4	6.1	0.4	2.5
F820-4-H2-IR	HLCALEEAFFGPSLFNSCQG	15.9	1.6	4.1	0.4	2.5
F820-4-F4-IR	HLCVLEERFWGASLFGQCSG	6.8	1.9	4.7	0.4	2.5
F820-4-B6-IR	QLCDLEELFWGASLFGYCPG	4.1	0.8	1.9	0.4	2.4
F820-4-B11-IR	HLCVLEERFWGASIWGSCSG	22.2	3.1	7.0	0.4	2.3
F820-4-H6-IR	QLCVLEELFWGGSLWGQCSR	4.1	1.1	2.4	0.5	2.2
F820-4-H9-IR	PLCVLEELFWGAAQFGQCSG	3.1	0.9	1.9	0.5	2.1
F820-4-D3-IR	QLCDLEERFWGVSLFGLCSG	4.6	1.3	2.5	0.5	1.9
F820-4-C1-IR	QLCVLEEVFWGASLFGLCTG	13.0	1.1	2.1	0.5	1.9
F820-4-D12-IR	QL.DLNTWSGLCLCSVTVRV	10.4	1.2	2.0	0.6	1.7
F820-4-B8-IR	DLCVLEESLWGKALFGYCSD	7.2	2.2	3.4	0.6	1.5
F820-4-C6-IR	HLCVLEEVFWGSSMFGDCSG	13.9	2.5	2.8	0.9	1.1
F820-4-C10-IR	HLCDLEELFWGASLFGDCQG	5.3	2.6	2.9	0.9	1.1
F820-4-D4-IR	QLCVLDALMWGGCRLGHQCG	3.5	2.3	2.1	1.1	0.9
F820-4-E1-IR	QLCVLEEKFWGTSLFGDCMG	1.6	1.6	1.5	1.1	0.9
F820-4-B3-IR	HLCVLEEVFWGAAQFGSCSG	15.9	0.6	5.0	1.2	0.8
F820-4-D2-IR	QLCVLEELFWGPSMFGYCSG	7.8	3.2	2.5	1.3	0.8
F820-4-C5-IR	HLCDLEELFWGASGFAQCYG	21.5	4.0	2.3	1.8	0.6

FIG. 4D-2

Clone Parental/Design	Sequence
	HLCVLEELFWGASLFGYCSG
F820-4-B5-IR	HLCMLEEQFWGASLFSRCSG
F820-4-A2-IR	TCAFWKNGSGVRRCSVTAVV
F820-4-E2-IR	PLCGLKN.SGVRLCSSPALV
F820-4-D10-IR	PLCLQEELFWGASLFGYCSG
F820-4-H7-IR	PLCDLEELFWGASLFGDCPG
F820-4-G6-IR	DLCVLEELFWDGSLFASCSG
F820-4-C2-IR	PLCVLEEQLWGTALFGSCTG
F820-4-B4-IR	PLCLVEELLWGASLFSQCTG
F820-4-C7-IR	PLCDLEELYWGAALFGSCSG
F820-4-F10-IR	GLCFLEEQFWGTSLFRDCPG
F820-4-G5-IR	PLCVVEELFWGASLYGQCSG
F820-4-F2-IR	RLCVLEELFWGASRFRGCSG
F820-4-H8-IR	PLCVLEELHWGAALFGYCSG
F820-4-D7-IR	NLCVVEELFWGASLFPNCSG
F820-4-B2-IR	QLCVLEELFWGASMFEDCSG
F820-4-C3-IR	HLCVLEEQFWGASLFGQCSG
F820-4-H4-IR	PLCVLEEIYWGAALFGDCYG
F820-4-B10-IR	PLCVLEELFWGLSLDKNCS
F820-4-A5-IR	QLCVLEELFWGASLFSGCSG
F820-4-F6-IR	PLCDLEALFWGESLFGGCSG
F820-4-F1-IR	HLCVLEEMFWGTSHFDGCSG
F820-4-A3-IR	DLCVLEELFWGAPLFGLCSG
F820-4-D1-IR	DLCVLEELFWGVALYGGCSG
F820-4-F5-IR	QLCVLEELYWGASLFGHCSG
F820-4-F12-IR	HLCVLEDRFWGASLFGPCSG
F820-4-A11-IR	HLCGMEEMFWGVALFRNCSG
F820-4-E8-IR	PLCVLEQLYWGESLFVYCSG
F820-4-H3-IR	HLCLLEELFWGEALWGYCSG

Ratios over Background			Comparisons	
E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
39.1	**1.8**	**27.7**	**0.1**	**15.4**
28.1	0.9	17.9	<0.1	21.1
34.0	1.6	22.7	0.1	13.9
21.3	0.7	9.0	0.1	13.4
34.1	1.0	12.1	0.1	12.1
14.2	0.6	6.5	0.1	11.6
14.0	0.5	6.1	0.1	11.5
38.1	1.2	11.8	0.1	9.9
15.1	0.7	6.4	0.1	8.7
46.3	2.7	22.2	0.1	8.2
14.5	0.6	4.7	0.1	8.0
8.8	0.6	4.4	0.1	7.5
11.7	0.6	4.2	0.1	7.4
16.0	0.6	4.7	0.1	7.3
14.5	0.8	5.9	0.1	7.1
5.0	0.4	2.4	0.2	6.9
37.5	1.1	7.5	0.2	6.6
21.2	1.1	6.4	0.2	5.9
7.5	0.7	3.7	0.2	5.6
5.3	0.8	4.4	0.2	5.2
5.7	0.6	3.0	0.2	4.9
9.1	1.0	4.7	0.2	4.7
5.9	0.8	3.5	0.2	4.5
25.7	2.3	10.5	0.2	4.5
3.7	0.6	2.7	0.2	4.2
11.3	0.6	2.2	0.3	3.5
7.6	0.8	2.7	0.3	3.5
8.0	1.2	4.3	0.3	3.5
17.5	2.6	9.0	0.3	3.4

FIG. 4D-1

Clone	Sequence	Ratios over Background			Comparisons	
Parental/Design	HLCVLEELFWGASLFGYCSG	E-Tag 39.1	IGFsR 1.8	IR 27.7	IGFR/IR 0.1	IR/IGFR 15.4
F815-4-F11-IGFR	PLCFLQELFGGASLGGYCSG	33.4	12.3	1.0	12.3	0.1
F815-4-E12-IGFR	FMCGLQELVGGAALLGHCSG	33.7	15.1	1.7	8.9	0.1
F815-4-H10-IGFR	PLCFLQELFGGGSLSGYCSG	30.1	8.5	1.0	8.5	0.1
F815-4-B7-IGFR	FLCGLEELAWGVSRSGYCFG	35.2	23.9	4.8	5.0	0.2
F815-3-B5-IGFR	PLCFLAELFSGSALGGDCSR	33.9	4.8	1.0	4.8	0.2
F815-4-D12-IGFR	PLCVLQELFGGGSLGGYCSG	33.6	7.0	1.8	3.9	0.3
F815-4-C11-IGFR	QLCVLE#LFWGACLFGYCAG	13.9	4.6	1.8	2.6	0.4
F815-4-C7-IGFR	FLCGLQELSGVASLFGQCSG	16.8	2.0	1.0	2.0	0.5
F815-4-E7-IGFR	RVCVLEQLVWGASLFGA*SG	26.9	3.8	1.9	2.0	0.5
F815-4-G7-IGFR	FYCGLEELSWGAALFGYCSG	30.4	9.0	5.0	1.8	0.6
F815-4-A10-IGFR	FLCGLEELSQGAVLFGHCYG	30.8	3.7	2.2	1.7	0.6
F815-3-B3-IGFR	HLCVLVGLFWDASLFGQCSG	7.6	1.0	2.0	0.5	2.0
F815-3-G1-IGFR	QRCIRAALFWCATLLGGCAG	20.5	1.0	2.0	0.5	2.0
F815-4-G12-IGFR	HQCIPDGMSQGAALRGNCSD	7.6	1.0	2.5	0.4	2.5
F815-3-H1-IGFR	HLCVLEDELWGVSLFGYCSS	18.4	1.0	6.8	0.1	6.8

FIG. 4C

Clone Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
F815-4-F11-IR	RLCVLEERFWGAALFGQCSG	31.8	1.0	33.7	<0.1	34.2
F815-3-A9-IR	PLCVLEELFWGASLFGQCSG	31.9	1.0	35.5	<0.1	34.1
F815-4-G11-IR	SLCVLEELFWGGSRFGQCSG	32.3	1.0	34.4	<0.1	33.9
F815-3-D8-IR	HLCLLEEQFWGASLFGYCFE	32.3	1.0	33.3	<0.1	33.7
F815-4-G4-IR	HLCVLEEQFWGASLFGQCSG	23.8	1.0	32.2	<0.1	33.7
F815-3-C8-IR	DLCLLEELLWGASRFGQCSG	33.9	1.0	35.1	<0.1	33.6
F815-4-G12-IR	YLCVLEERFWGASLFGQCSG	31.7	1.0	33.5	<0.1	33.5
F815-3-D12-IR	HLCVLEEQFWGASLFGSCSG	33.3	1.0	34.8	<0.1	33.4
F815-4-F7-IR	QLCVLEEQLWGASLFGQCSG	33.3	1.0	34.3	<0.1	33.4
F815-4-F2-IR	HLCVLEELF*GESLFGYCSG	26.1	1.0	33.8	<0.1	33.3
F815-3-B9-IR	HLCVLEELFWGASLFGQCSG	33.6	1.1	35.7	<0.1	33.2
F815-4-H2-IR	PLCVLEELFWGASHFGQCSG	36.1	1.2	38.4	<0.1	33.0
F815-4-E11-IR	HLCVLEELVWGASLFGQCAG	33.2	1.1	35.4	<0.1	33.0
F815-4-G1-IR	QLCVLEELIWGASLFGQCAG	27.9	1.0	31.5	<0.1	32.8
F815-3-A11-IR	HLCGLEELFWGASLFGQCSG	37.7	1.2	40.1	<0.1	32.7
F815-4-F6-IR	HLCVLEELVWGESLFGQCSG	32.3	1.1	34.6	<0.1	32.6
F815-3-D9-IR	RLCVLEELYWGASLFGQCSG	31.4	1.0	32.5	<0.1	32.5
F815-3-C11-IR	RLCILEELFWGASLFGQCSG	33.4	1.1	35.7	<0.1	31.9
F815-4-G2-IR	HLCVLEELFWGATLFDQCSG	30.2	1.1	34.3	<0.1	31.4
F815-3-C9-IR	HLCFLEELFWGASMFGQCSG	29.7	1.0	31.4	<0.1	31.0
F815-4-H10-IR	HLCIVEELFWAAPLFGQCSG	31.9	0.9	27.6	<0.1	29.4
F815-4-F3-IR	HLCVLEELWWGASLFAQCSA	19.4	1.0	28.0	<0.1	28.9
F815-4-F5-IR	NLCALEELFWGASQFRYCPG	12.3	0.9	24.8	<0.1	26.8
F815-4-H1-IR	RLCVLEELFWGASLFGQCSG	6.9	1.0	15.8	0.1	16.5
F815-4-E5-IR	PLCVLEELFWGASLFGQCPG	3.5	1.0	13.6	0.1	14.0
F815-4-H5-IR	NLCVLEELFWGASLFGQCSG	5.5	1.0	13.1	0.1	13.5
F815-3-C10-IR	QLCVLG#RFWGGSLCGYCSD	3.5	1.1	5.2	0.2	4.5

FIG. 4B-3

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
F815-3-A6-IR	QLCVLEELFWGSSLFGQCSG	34.6	1.1	39.0	<0.1	36.2
F815-3-D3-IR	DLCVVEELFWGKSLFGQCSG	33.8	1.0	36.2	<0.1	36.2
F815-3-B12-IR	DLCVLEELFWGSSLFGQCSG	33.2	1.0	35.7	<0.1	36.2
F815-4-G10-IR	YLCVLEEQFWGASLFRQCFG	35.4	1.0	37.2	<0.1	36.1
F815-4-E3-IR	HLCVLEELLWGSSLFGQCSG	32.4	1.0	35.0	<0.1	36.1
F815-4-E6-IR	PLCGLEELFWGASLFGQCSD	33.2	1.0	34.5	<0.1	36.1
F815-4-F1-IR	HLCVLEELFWGSSLFAQCSG	29.4	0.9	32.5	<0.1	36.0
F815-4-G8-IR	PLCAIEELFWGAALFGQCSG	36.8	1.1	38.2	<0.1	35.9
F815-4-H12-IR	HLCVLEEQFWGASLFGDCSG	30.5	0.9	31.9	<0.1	35.9
F815-4-G3-IR	PLCVLEELFWGAPLFGQCSD	31.4	1.0	35.7	<0.1	35.7
F815-3-C2-IR	DLCGLEELFWGAALFGQCTS	32.3	1.0	36.1	<0.1	35.6
F815-4-E10-IR	QLCVLEKQLWGASLFWQCSG	35.4	1.0	36.5	<0.1	35.4
F815-3-A12-IR	HLCVLEELFWGASLYGQCPG	32.1	1.0	36.3	<0.1	35.3
F815-3-B8-IR	HLCVLEELFWGASLFDQCSG	33.6	1.0	35.8	<0.1	35.3
F815-3-B2-IR	HLCVLEELLWGASLFGQCSG	31.0	1.0	35.3	<0.1	35.3
F815-3-C3-IR	PLCVLEELFWGVSLFGQCGG	30.1	1.0	35.3	<0.1	35.3
F815-3-A7-IR	HLCVLEELFWGASQWGQCSG	33.1	1.0	35.8	<0.1	35.2
F815-4-F9-IR	RLCVLEEQFWGGALFGQCSG	33.4	1.0	35.7	<0.1	35.2
F815-3-B7-IR	QLCVLEELFWGVSLFAQCSG	32.0	1.0	33.5	<0.1	35.0
F815-4-E4-IR	HLCVLEELFWGAALFGQCFG	28.0	1.0	33.4	<0.1	35.0
F815-4-E12-IR	YLCVLEELFWGASQFGQCSG	28.0	0.9	30.2	<0.1	34.8
F815-4-F8-IR	HLCVLEELYWGASLFGQCSG	33.8	1.0	35.2	<0.1	34.7
F815-3-C7-IR	HLCVLEERFWGVSLFGQCSG	33.9	1.0	34.7	<0.1	34.7
F815-4-F10-IR	PLCVLEELFWGASRFGQCSG	32.7	1.0	34.2	<0.1	34.7
F815-3-D11-IR	HLCVLEDLFWGASLFDQCSG	35.4	1.1	37.3	<0.1	34.6
F815-4-E7-IR	HLCDLEVLFWGASLFGQCSG	30.3	0.9	32.2	<0.1	34.6
F815-3-A10-IR	QLCILEEQFWGTSLFGYCSG	34.0	1.1	36.4	<0.1	34.3
F815-3-B11-IR	ALCVLEELFWGESLFGQCSG	33.7	1.1	36.3	<0.1	34.2

FIG. 4B-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
F815-4-H9-IR	PLCVLEELFWSTPLFGQCSY	34.9	0.9	37.6	<0.1	40.8
F815-3-B1-IR	HLCVLEELFWGASLFAQCVG	31.7	0.9	35.8	<0.1	39.3
F815-3-D1-IR	DLCVLEELFWGASRFGQCSG	30.4	0.9	33.5	<0.1	38.9
F815-3-D4-IR	HLCVLEELFWGASLFGQCAG	31.5	0.9	33.6	<0.1	38.8
F815-3-C5-IR	HLCVVEELFWGASLFGQCSG	31.1	0.8	31.2	<0.1	38.5
F815-4-H3-IR	NLCDLEVLFWGASLFRQCSG	33.7	1.0	37.2	<0.1	38.4
F815-3-A5-IR	PLCVLEEQFWGASLFGQCSG	37.4	1.1	40.9	<0.1	38.3
F815-3-D7-IR	QLCVLEELFWGASEFGQCSG	33.6	0.9	34.3	<0.1	38.3
F815-3-A1-IR	HLCELEELFWGASLFGQCSG	29.8	0.9	34.8	<0.1	38.0
F815-4-H4-IR	PLCVLEELFWGESLFGQCSG	31.1	0.9	32.7	<0.1	38.0
F815-3-A3-IR	HLCVLEELFWGASRFGQCSG	32.8	1.0	39.1	<0.1	37.9
F815-3-B3-IR	KLCVLEELFWGASLFGQCSG	33.7	1.0	37.5	<0.1	37.5
F815-3-A4-IR	YLCVLEELSWGASLFGQCSG	32.5	1.0	36.9	<0.1	37.5
F815-3-D2-IR	HLCVLEELLWGASLFAQCSG	31.9	0.9	34.1	<0.1	37.4
F815-3-C4-IR	QLCVLEQLFWGESLFGQCSG	31.6	0.8	31.8	<0.1	37.4
F815-3-B4-IR	HLCVLEELFWGGNLFSQCSG	33.8	1.0	36.7	<0.1	37.3
F815-3-C1-IR	HLCVLEELFWGASLYGQCSG	29.0	0.9	35.0	<0.1	37.3
F815-4-G9-IR	SLCALEEQFWGAALFGYCSG	36.5	1.0	38.9	<0.1	37.1
F815-4-G6-IR	HLCVLEEQFWGASLFDGCAG	34.9	1.0	36.4	<0.1	37.0
F815-3-A8-IR	QLCVLEELFWGASLFGQCSG	34.7	1.1	39.3	<0.1	36.9
F815-4-G5-IR	PLCVLEELFWGAALFGQCSG	26.5	1.0	35.1	<0.1	36.8
F815-3-B5-IR	HLCVLEELFWGASLFGQCTG	33.2	0.9	34.1	<0.1	36.8
F815-4-F4-IR	PLCVLEELFWGGSLFGQCSG	28.6	0.8	30.0	<0.1	36.7
F815-3-A2-IR	QLCVLEELVWGASLFGQCSG	32.5	1.0	36.6	<0.1	36.6
F815-3-B6-IR	HLCVVEELIWGASLFGQCSR	31.6	0.9	32.9	<0.1	36.5
F815-4-H7-IR	DLCVLEELFWGASLFGQCAG	33.7	1.0	37.6	<0.1	36.4
F815-4-H8-IR	QLCVLEERFWGASLFGQCSG	35.8	1.0	37.0	<0.1	36.4
F815-4-G7-IR	NLCVLEELFWGAALFGQCSG	33.7	1.0	35.8	<0.1	36.3

FIG. 4B-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXLXXLXXYFXXXXXX	--	--	--	--	--
rB6-3-F1-IGFR	NTILGDLWRYFAGSGGM	26.5	5.8	1.4	4.1	0.2
rB6-4-B7-IGFR	?DVLKKL?VYFELSGGA	31.1	11.4	2.9	3.9	0.3
rB6-4-C10-IGFR	GGPLQGLYTYFKQSPVC	32.2	3.7	1.0	3.7	0.3
rB6-3-A1-IGFR	DRLLSGLWAYFAGNGGS	21.1	3.5	1.0	3.5	0.3
rB6-3-F6-IGFR	DLILQSLLDYFQGRPVG	25.1	3.5	1.0	3.5	0.3
rB6-3-H5-IGFR	LALLPMLWDYFVATDPQ	35.5	18.1	5.6	3.2	0.3
rB6-4-D8-IGFR	DSILRELRDYFARTHIA	36.2	22.5	7.5	3.0	0.3
rB6-4-A8-IGFR	DGVLGQLWQYFAQYPGS	41.1	30.6	10.6	2.9	0.3
rB6-4-H8-IGFR	?PPLDALWEYFTGTARD	38.7	33.0	11.5	2.9	0.3
rB6-3-E2-IGFR	DNVLEGLWSYFALWSQL	20.9	2.2	1.0	2.2	0.5
rB6-3-C2-IGFR	SAVLEYLLAYFARTGAA	31.0	2.1	1.0	2.1	0.5
rB6-4-G8-IGFR	DRALGPLWRYFMVNNGQ	38.7	5.5	2.6	2.1	0.5
rB6-3-G5-IGFR	WRILDRLLAYFKESQGD	32.8	2.0	1.0	2.0	0.5
rB6-4-C9-IGFR	DDVLVTLFQYFRASTGV	37.6	30.2	15.1	2.0	0.5
rB6-4-D11-IGFR	FDVLTWLGRYF*MNTGK	36.6	5.5	3.0	1.8	0.5
rB6-4-B11-IGFR	RDVLDGLREYFRASVGG	25.2	4.2	2.4	1.8	0.6
rB6-4-E11-IGFR	IKTLNDLLAYFRGDLDV	38.1	29.8	22.2	1.3	0.7
rB6-3-G3-IGFR	DEALLWLMRYFRGSPSP	31.6	8.7	7.2	1.2	0.8
rB6-4-H12-IGFR	ESPLDALRAYFSGRRNW	40.1	2.8	2.5	1.1	0.9
rB6-4-G12-IGFR	IQSL*DLLQYFVSSPSV	36.7	32.5	31.4	1.0	1.0
rB6-3-C4-IGFR	GGILD?LQDYFRSTDVG	37.1	6.2	13.5	0.5	2.2

FIG. 3D-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20β-4-F8-IR	HLCVLEELFWGASLFGYCSG	39.1	1.8	27.7	0.1	15.4

FIG. 4A

Cl ne Design	Sequence XXXLXXLXXYFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
rB6-4-B10-IGFR	RPVLGWLFDYFVASDPM	33.1	26.9	1.0	26.9	<0.1
rB6-3-E6-IGFR	RWPLSALMDYFRRSDGV	37.6	26.6	1.0	26.6	<0.1
rB6-4-B9-IGFR	DGVLASLWRYFVSGGTL	39.2	26.3	1.0	26.3	<0.1
rB6-3-F5-IGFR	DRQLGWLWDYFHLTDLP	33.2	15.6	1.0	15.6	0.1
rB6-3-B6-IGFR	DGILGLLMAYFVES?RV	37.4	13.3	1.0	13.3	0.1
rB6-3-D4-1GFR	QDLLGRLWLYFAETDTV	31.2	20.7	2.0	10.4	0.1
rB6-4-D10-IGFR	SGVLADLFRYFQRHPWP	31.7	10.1	1.0	10.1	0.1
rB6-3-D6-IGFR	DPPLGGLWTYFSRSDPG	33.9	9.9	1.0	9.9	0.1
rB6-4-F9-IGFR	DSVLRSLYSYFASGDIA	34.3	28.3	3.0	9.4	0.1
rB6-3-E1-IGFR	DGVLAALEAYFRHGPRD	30.5	9.3	1.0	9.3	0.1
rB6-3-B2-IGFR	DEILGALYSYFSLSGGA	22.2	8.8	1.0	8.8	0.1
rB6-3-D7-IGFR	QDVLGALQRYFASGEPW	31.2	7.6	1.0	7.6	0.1
rB6-4-C11-IGFR	DSVLQYLLNHFGADSKQ	33.7	7.6	1.0	7.6	0.1
rB6-4-F12-IGFR	NEVLEGLFSYFVY?ANG	38.1	7.3	1.0	7.3	0.1
rB6-4-F7-IGFR	SGILGQLLRYFKGAGGG	38.6	7.3	1.0	7.3	0.1
rB6-3-G6-IGFR	DELLDRLWQYFQVGGDL	34.0	7.1	1.0	7.1	0.1
rB6-4-E8-IGFR	PGILLDLWRYFASAPDQ	37.6	6.9	1.0	6.9	0.1
rB6-4-G10-IGFR	DSVLLDLYEYFSSGSSG	34.9	14.5	2.2	6.6	0.2
rB6-4-B12-IGFR	DGMLSRLWEYFAGTNVP	36.3	28.9	4.5	6.4	0.2
rB6-3-B5-IGFR	DVILGGLWDYFASGGGH	17.2	6.1	1.0	6.1	0.2
rB6-3-C5-IGFR	GGVLAALERYFRVSAGD	38.7	15.8	2.9	5.4	0.2
rB6-4-B8-IGFR	DEVLGRLWAYFAQESLG	31.9	22.0	4.1	5.4	0.2
rB6-3-H2-IGFR	DGILQSLWDYFARSPVG	31.8	22.4	4.2	5.3	0.2
rB6-3-E5-IGFR	VDILSELWDYFRRGEEG	37.0	20.5	4.0	5.1	0.2
rB6-3-B3-IGFR	DKVLRLLGEYFATHSKG	31.7	4.8	1.0	4.8	0.2
rB6-4-G7-IGFR	QGPLAWLRDYFASGTRS	37.4	10.0	2.1	4.8	0.2
rB6-3-A3-IGFR	QDVLRSLLSYFMGNGDV	27.2	4.7	1.0	4.7	0.2
rB6-4-E9-IGFR	DGVLSKLWEYFKIQGND	37.3	20.1	4.8	4.2	0.2

FIG. 3D-1

Cl ne	Sequence IR/IGFR	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
Design	XXXLXXLXXYFXXXXXX	--	--	--	--	--
rB6-4-E7-IR	LDPLDALLQYFWSVPGH	26.4	1.0	15.5	0.1	15.5
rB6-4-A12-IR	LDALDRLMRYFEERPSL	34.9	1.0	12.0	0.1	12.0
rB6-3-E6-IR	ADELEWLLDYFMHQPRP	9.0	1.0	4.8	0.2	4.8
rB6-4-E11-IR	DQELGWLRGYFEWTARD	31.2	1.6	5.9	0.3	3.7
rB6-4-F12-IR	DGVLEELFSYFSATVGP	30.4	1.0	3.4	0.3	3.4
rB6-4-D11-IR	PMNLSELWDYFRLKPGR	41.9	15.7	30.2	0.5	1.9
rB6-4-A8-IR	DSILRELRDYFAPYSHC	25.6	2.4	4.6	0.5	1.9
rB6-4-E8-IR	DDALEWLLNYFQNGHVQ	33.0	9.7	15.9	0.6	1.6
rB6-4-B9-IR	GDILDALLRYFEFGVDT	42.7	17.2	21.7	0.8	1.3
rB6-3-A6-IR	GDQLAWLLAYFQSDGSD	32.3	2.9	2.8	1.0	1.0
rB6-4-C7-IR	DGVLEGLLSYFTSTNSH	31.4	2.6	2.3	1.1	0.9
rB6-4-H12-IR	ARPLDWLLDYFKQGARG	26.0	10.0	7.2	1.4	0.7
rB6-3-C6-IR	DDMLRQLWLYFEASAGG	34.2	19.1	12.8	1.5	0.7
rB6-4-G12-IR	DPWLAWLGRYFGETATG	37.7	6.1	3.1	2.0	0.5
rB6-4-G12-IR	DPTLFGLLRYFQESGIA	33.3	7.6	3.5	2.2	0.5
rB6-4-C11-IR	MDPLRGLLMYFSQGGLV	26.6	18.7	4.7	4.0	0.3
rB6-4-G8-IR	DGLLWQLWDYFALSEHR	37.3	7.4	1.3	5.7	0.2
rB6-4-B8-IR	DNWLSALMAYFMGSGES	31.1	28.6	1.0	28.6	<0.1
rB6-4-D7-IR	DDVLNYLLGYFRQSDGL	24.1	29.4	1.0	29.4	<0.1

FIG. 3C

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20-4-F9-IGFR	PLAELWAYFEHSEQGRSSAH	33.1	19.3	1.0	19.3	0.1
R20-4-H4-IGFR	PVLSGLLRYFAGGPLGQPQS	24.1	5.6	3.2	1.8	0.6
R20-4-F9-IGFR	GGYLDDLWHYFRDGQALQPW	2.5	2.4	1.4	1.7	0.6
R20-4-D6-IGFR	VDQRQGGWLLALENYFRSTV	6.1	2.9	1.9	1.5	0.7
R20-4-G2-IGFR	DVPAGGLLRQMWVYFRDSDP	6.3	2.2	2.0	1.1	0.9

FIG. 3A

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXCXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
20C-3-F3-IGFR	RRVACTQADGLLCESDPLKALLSYF	35.5	32.8	17.9	1.8	0.5

FIG. 3B

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	E-Tag --	IGFR --	IR --	IGFR/IR --	IR/IGFR --
NNRPβ-4-H11-IR	DYTAFYGYFNNLLRTSG	2.3	12.4	9.0	1.4	0.7
NNRPα-1-B1-IR	FQSSFYGYFESLLMSYK	18.8	18.7	11.5	1.6	0.6
NNRPα-2-D7-IR	DTNAFYRYFEGLLWSEH	21.0	21.8	13.2	1.6	0.6
NNRPα-2-D8-IR	GGSSFYRYFEQLLAQWE	20.2	19.8	12.2	1.6	0.6
NNRPα-3-E1-IR	SQGGFYRYFEKLLDEVT	20.0	20.5	12.9	1.6	0.6
NNRPα-3-E5-IR	RSGLFYRYFEELLQGAI	20.0	24.5	15.5	1.6	0.6
NNRPα-3-H3-IR	QGGGFYHYFLSLLEEVG	19.8	19.1	12.2	1.6	0.6
NNRPα-3-H5-IR	WRGAFYRYFQTLLSDEG	19.9	18.0	11.1	1.6	0.6
NNRPα-1-A3-IR	AAGFYGYFYSLLGDQT	24.4	14.9	7.9	1.9	0.5
NNRPα-3-E6-IR	RNSGFYRYFQHLVSEWE	23.1	19.0	9.6	2.0	0.5
NNRPα-3-F9-IR	QHRLFYSYFAELLGRDT	21.1	18.8	9.6	1.9	0.5
NNRPα-3-G6-IR	QIDEFYRYFADQLRGFA	22.4	17.7	9.0	2.0	0.5
NNRPα-3-H9-IR	LGGGFYRYFNLLVMGSG	18.3	13.1	6.9	1.9	0.5
NNRPα-1-A8-IR	GDRAFYRYFQRQLEGWG	16.9	13.8	5.7	2.4	0.4
NNRPα-1-A9-IR	CEDAFYRYFVNLLGQGC	16.5	15.2	5.6	2.7	0.4
NNRPα-2-D6-IR	NYSQFYRYFEMLLEGDV	19.4	18.5	6.8	2.7	0.4
NNRPα-3-F6-IR	VGDAFYRYFQGLLRQDQ	22.8	19.5	7.9	2.5	0.4
NNRPα-3-H2-IR	MHGSFYRYFQDLLQAPP	19.9	18.9	8.5	2.2	0.4
NNRPα-1-B6-IR	DVGDFYRYFGLLLTSDR	14.1	11.5	3.9	3.0	0.3
NNRPα-2-C6-IR	NSAAFYGYFSQLLAQIR	18.4	19.2	4.1	4.7	0.2
NNRPγ-4-A1-IR	IIGGFYSYFNSVLRLGT	9.7	10.9	1.8	6.0	0.2
NNRPγ-4-A7-IR	RFDPFYSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2
NNRPγ-4-A9-IR	EGSGFYGYFFSLLGLQG	3.0	10.0	1.4	7.1	0.1
NNRPγ-4-B11-IR	LKDGFYDYFWQRLHLGS	4.1	18.7	1.2	15.5	0.1

FIG. 2P-5

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NNRPγ-4-A12-IR	AFYRYFRDLLFSGF	4.9	16.3	14.9	1.1	0.9
NNRPγ-4-B8-IR	DDRGFYRYFESLLLGSS	6.1	21.3	19.9	1.1	0.9
NNRPα-1-A5-IR	LSTSFYQYLAGLLRGDR	2.3	1.4	1.1	1.2	0.8
NNRPα-1-B7-IR	GSSGFYRYFNMLMLSQT	19.2	15.7	12.4	1.3	0.8
NNRPα-2-C7-IR	GDRGFYRYFEGLLASVG	19.6	20.0	16.5	1.2	0.8
NNRPα-2-C11-IR	NSAAFYRYFEQLLEREV	20.1	20.0	16.3	1.2	0.8
NNRPα-2-C12-IR	LSDGFYRYFEQLMGARS	14.3	10.1	8.5	1.2	0.8
NNRPα-2-D12-IR	RSTLFYRYFQNLLEEVG	11.5	11.4	9.3	1.2	0.8
NNRPα-3-G2-IR	TRGGFYRYFEDLLQVYS	20.8	20.7	16.1	1.3	0.8
NNRPα-3-G8-IR	GVSGFYRYFQSLLDSYG	14.7	11.0	9.2	1.2	0.8
NNRPα-3-G10-IR	QNDAFYSYFNSLLQAYT	18.8	16.5	13.9	1.2	0.8
NNRPα-3-G11-IR	RQQDFYRYFRQLLLEEV	12.0	10.3	8.5	1.2	0.8
NNRPα-3-G12-IR	EGSGFYRYFEKLLLQSP	11.7	11.8	9.3	1.3	0.8
NNRPγ-4-B2-IR	RHKAFYRYFEELLQKNV	22.8	30.3	25.3	1.2	0.8
NNRPα-1-B8-IR	GRMTRLIVRSTVISRELLHYSL	16.1	10.1	6.9	1.5	0.7
NNRPα-2-C5-IR	QALSFYRYFERLLDEVS	18.1	19.2	13.7	1.4	0.7
NNRPα-2-C9-IR	SKSAFYRYFDELLGNSG	22.9	21.7	16.1	1.3	0.7
NNRPα-2-D2-IR	LGGAFYRYFAQLLNSHV	26.1	26.2	17.6	1.5	0.7
NNRPα-2-D5-IR	LNSGFYGYFVQLLSGHQ	21.7	21.1	15.4	1.4	0.7
NNRPα-2-D11-IR	SQSSFYRYFESLLEDNP	12.3	10.8	7.8	1.4	0.7
NNRPα-3-E2-IR	ADGGFYGYFAALLGSVS	24.4	25.5	18.3	1.4	0.7
NNRPα-3-E4-IR	QNGSFYRYFIALLGDSG	23.0	22.3	14.7	1.5	0.7
NNRPα-3-F4-IR	WDTGFYRYFIELLEDRD	24.9	25.1	17.6	1.4	0.7
NNRPα-3-G4-IR	HPRDFYRYFERLLNQVD	20.9	20.4	14.1	1.5	0.7
NNRPα-3-H4-IR	DGGAFYRYFMDLLGAHE	17.7	17.6	11.6	1.5	0.7
NNRPα-4-E12-IR	AGRGFYRYFEHLLAGRE	4.3	15.4	10.8	1.4	0.7
NNRPβ-4-G11-IR	SSRGFYRYFRELLADSW	6.6	18.4	13.1	1.4	0.7
NNRPβ-4-H6-IR	KYSGFYEYFNALLGRRE	2.2	16.1	11.7	1.4	0.7

FIG. 2P-4

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	E-Tag --	IGFR --	IR --	IGFR/IR --	IR/IGFR --
NNRPγ-4-A10-IR	SGFAFYQYFQELLAGHD	7.6	20.3	22.0	0.9	1.1
NNRPγ-4-B6-IR	GDGGFYGYFASLLSGEG	12.2	22.3	24.2	0.9	1.1
NNRPγ-4-B9-IR	EANGFYRYFYDLLQDFG	6.7	22.9	25.9	0.9	1.1
NNRPα-4-C8-IR	AVNGFYRYFNRLLESVE	8.5	16.3	16.0	1.0	1.0
NNRPα-4-C9-IR	QQDGFYRYFLDLLDEVA	5.6	20.7	19.9	1.0	1.0
NNRPα-4-C10-IR	ISQGFYGYFSRLLQDTE	6.7	16.5	17.2	1.0	1.0
NNRPα-4-E11-IR	YSTGFYRYFLDLLDGMP	6.0	20.3	20.9	1.0	1.0
NNRPβ-4-F11-IR	PNGDFYRYFLDLLGSVG	7.7	21.8	21.9	1.0	1.0
NNRPβ-4-G2-IR	RHQAFYSYFRDLPRECP	19.1	24.7	25.6	1.0	1.0
NNRPβ-4-G9-IR	ETEGFYRYFEELLAQVA	7.8	27.3	26.4	1.0	1.0
NNRPβ-4-H7-IR	AGDRFYDYFDRLLADYD	2.6	26.6	27.9	1.0	1.0
NNRPβ-4-H8-IR	GGSGFYRYFWGLLAEQE	3.6	23.0	24.1	1.0	1.0
NNRPγ-4-B1-IR	LLNRLYRYFAGAEGWFG	17.6	24.5	23.4	1.0	1.0
NNRPγ-4-B10-IR	DGSGFYRYFEMLLGSGL	5.5	18.3	19.0	1.0	1.0
NNRPα-1-B3-IR	RDMAFYRYFSHLLESFQ	16.4	13.4	12.7	1.1	0.9
NNRPα-2-C2-IR	GNAGFYRISRILWQGTE	22.5	24.4	21.3	1.1	0.9
NNRPα-2-C3-IR	GNAGFYRYFADLMAGYE	19.6	21.7	19.7	1.1	0.9
NNRPα-2-D10-IR	YQAAFYRYFATLLSTTD	17.8	6.3	5.4	1.2	0.9
NNRPα-3-E11-IR	GGLGFYRYFQLLLGSSG	12.9	10.8	9.6	1.1	0.9
NNRPα-3-F5-IR	DGSGFYGYFDFVLRQFE	25.1	18.3	17.0	1.1	0.9
NNRPα-3-F8-IR	VGSGFYRYFDQLLGMYG	22.2	15.7	13.9	1.1	0.9
NNRPα-3-F10-IR	YGTDFYLYFDQLLLQYG	20.5	14.6	13.1	1.1	0.9
NNRPα-3-G7-IR	FNSSFYLYFRDLLNTVG	21.0	18.3	15.6	1.2	0.9
NNRPα-4-C2-IR	RAAGFYRYFEDLLGARG	25.5	25.1	23.3	1.1	0.9
NNRPα-4-D12-IR	TGAGFYRYFIDLLGETG	14.7	19.7	18.5	1.1	0.9
NNRPβ-4-G3-IR	RDLEFYGYFQELLRLNF	14.6	27.8	25.7	1.1	0.9
NNRPβ-4-G4-IR	GMGPFYRYFIDLLRESD	20.0	28.6	24.9	1.1	0.9
NNRPγ-4-A5-IR	HGDGFYQYFMEVLRLQN	17.0	29.0	27.3	1.1	0.9

FIG. 2P-3

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	Ratios over Background E-Tag --	IGFR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
NNRPβ-4-G10-IR	SSREFYSYFSGLLTTAL	8.8	8.7	11.7	0.7	1.3
NNRPβ-4-H2-IR	TGRGFYRYFEGLLEDWM	4.9	19.9	25.3	0.8	1.3
NNRPα-4-C1-IR	SGSWFYRYFEELLLQSG	15.5	18.0	21.1	0.9	1.2
NNRPα-4-C5-IR	GRGGFYQYFLDLLQTEA	18.0	23.3	26.9	0.9	1.2
NNRPα-4-C6-IR	GQNGFYRYFDTLLADWV	7.8	13.6	15.7	0.9	1.2
NNRPα-4-C12-IR	FAGSFYRYFEQLLLSEQ	12.3	16.7	19.9	0.8	1.2
NNRPα-4-D7-IR	DPNAFYRYFEGLLWREH	10.2	23.7	27.9	0.8	1.2
NNRPα-4-D11-IR	?GLNFYRYFVGLLTDTL	5.4	19.3	22.3	0.9	1.2
NNRPβ-4-F1-IR	RHINFYGYFDDLLATWH	21.7	23.0	28.6	0.8	1.2
NNRPβ-4-F9-IR	FHRGFYRYFINLLSGDA	10.1	18.4	22.5	0.8	1.2
NNRPβ-4-F12-IR	MGSSFYRYFETLLGQGL	4.5	13.5	16.6	0.8	1.2
NNRPγ-4-A3-IR	GSLDFYSYFWERLGLGP	16.4	22.3	26.8	0.8	1.2
NNRPα-1-A7-IR	STVSFYRYFYALLQSPC	16.9	1.2	1.3	0.9	1.1
NNRPα-4-C11-IR	LGGYFYRYFEDLLNHQS	7.8	19.7	21.2	0.9	1.1
NNRPα-4-D8-IR	DHRGFYRYFLYQLAGNV	6.9	17.6	20.1	0.9	1.1
NNRPα-4-D10-IR	EYSGFYGYFNHLLGSLG	6.4	17.2	19.5	0.9	1.1
NNRPα-4-E5-IR	TSNWFYQYFTDLLAGED	13.2	26.1	27.6	0.9	1.1
NNRPα-4-E8-IR	SSGGFYRYFSQLLTEMN	8.7	22.9	24.2	0.9	1.1
NNRPα-4-E10-IR	VHGEFYRYFESLLRETF	3.5	12.4	13.2	0.9	1.1
NNRPβ-4-F8-IR	SDEGFYRYFAQLLYGVT	8.1	22.9	25.2	0.9	1.1
NNRPβ-4-F10-IR	ETGGFYGYFQALLATYH	5.3	17.9	19.1	0.9	1.1
NNRPβ-4-G8-IR	GDRGFYRYFEWLLNDFG	10.6	27.2	28.9	0.9	1.1
NNRPβ-4-H3-IR	FGGAFYRYFEALLGEMG	3.9	24.2	25.7	0.9	1.1
NNRPβ-4-H9-IR	DGGAFYRYFEALLGELD	4.1	26.5	29.3	0.9	1.1
NNRPβ-4-H10-IR	WHSDFYRYFLSLLQEDG	3.4	22.3	24.6	0.9	1.1
NNRPγ-4-A6-IR	EEEGFYGYFYRLLGVER	14.9	25.8	27.6	0.9	1.1
NNRPγ-4-A8-IR	MDAGFYGYFSDLLANWG	9.8	22.8	24.7	0.9	1.1

FIG. 2P-2

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	Ratios over Background			Comparisons	
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
NNRPβ-4-G6-IR	RWPNFYGYFESLLTHFS	10.1	1.9	20.1	0.1	10.6
NNRPβ-4-F3-IR	HYNAFYEYFQVLLAETW	8.6	1.3	13.6	0.1	10.5
NNRPα-2-C1-IR	EGWDFYSYFSGLLASVT	19.7	2.0	10.9	0.2	5.3
NNRPα-4-E1-IR	LDRQFYRYFQDLLVGFM	11.5	6.5	21.2	0.3	3.2
NNRPα-3-H6-IR	WGRSFYRYFETLLAQGI	19.1	2.1	6.0	0.3	2.9
NNRPβ-4-F7-IR	RREGFYHYFQSLLDEYG	0.7	0.9	2.3	0.4	2.7
NNRPα-2-D1-IR	GGGQFYRYFIDMLVLDI	18.4	1.5	3.7	0.4	2.5
NNRPα-1-A1-IR	PTGPFDRYFARRLVWRG	15.2	1.3	3.1	0.4	2.4
NNRPα-2-C10-IR	RGGAFYRYFEGLLSQHN	18.8	3.8	8.8	0.4	2.3
NNRPα-3-G1-IR	WRDPFYRYFQDLLEGER	18.9	4.2	8.6	0.5	2.1
NNRPα-4-C3-IR	WGGEFYRYFVQLLSSED	17.9	12.9	25.7	0.5	2.0
NNRPα-4-D1-IR	GRESFYGYFLDLLQETV	16.2	12.7	23.2	0.5	1.8
NNRPβ-4-F4-IR	GHAEFYGYFQGLLDSYL	19.5	16.0	25.6	0.6	1.6
NNRPα-1-B2-IR	GGEAFYRYFWGLLTEWE	14.8	8.4	12.9	0.7	1.5
NNRPα-1-B4-IR	LSSGFYRYFTGLLSDGQ	19.1	6.3	9.2	0.7	1.5
NNRPα-4-D9-IR	DPGAFYRYFAQLMDTWN	7.6	16.9	25.7	0.7	1.5
NNRPβ-4-F2-IR	KHEQFYEYFRNLLGAMS	21.6	20.9	30.8	0.7	1.5
NNRPβ-4-H12-IR	RDGAFYRYFEDLLIAVD	5.2	13.8	20.0	0.7	1.5
NNRPα-4-E7-IR	RGNRFYEYFEYLLRDYG	9.4	21.9	29.7	0.7	1.4
NNRPα-1-B5-IR	ELGDFYRYFQLLLADWH	14.1	5.4	7.1	0.8	1.3
NNRPα-4-C4-IR	AQDAFYSYFSVLLGEHL	17.6	17.6	22.3	0.8	1.3
NNRPα-4-C7-IR	IGVNFYRYFEKLLDEF	4.5	11.2	14.9	0.8	1.3
NNRPα-4-D3-IR	TDSQFYSYFESLLETFG	16.4	13.5	17.9	0.8	1.3

FIG. 2P-1

Clon	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYXXhXXhhXXXXXX	—	— —	--	—	—
20E2B-3-D3-IGFR	CGSRRDFYGGIICLLGQKGVV	21.0	16.1	2.6	6.2	0.2
20E2B-4-H8-IGFR	PAGPCGFYCGLGLLLHGDQSP	7.2	5.3	0.9	5.9	0.2
20E2B-3-E9-IGFR	QAAPQDFYQGLWLLIHRDPTM	14.7	16.2	2.8	5.8	0.2
20E2B-4-H9-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	0.9	5.6	0.2
20E2B-1-A8-IGFR	TLRSPTFYDWLEMVLTHGQGG	16.1	4.4	0.9	5.0	0.2
20E2B-4-H11-IGFR	STHSRAFYDAIAQLVGSVLGP	10.7	11.0	2.3	4.8	0.2
20E2B-3-C9-IGFR	RQGGGSFYELLCGLVGGEVCV	17.9	19.7	4.2	4.6	0.2
20E2B-3-E6-IGFR	RQQASGFYRALHDLMLRTQDY	24.5	21.6	4.7	4.6	0.2
20E2B-3-E11-IGFR	SRANNLFYMGLSQLLRDNRGL	16.5	7.7	1.9	4.1	0.2
20E2B-4-G8-IGFR	GRALDPFYDQLRDLVARSGGG	11.1	14.9	3.7	4.1	0.2
20E2B-4-H10-IGFR	EASCRTFYCGLMALIGGDDQR	2.2	2.5	0.8	3.1	0.3
20E2B-3-E7-IGFR	QNGCKDFYCLIDNLIRYGPGG	14.4	8.8	3.0	3.0	0.3
20E2B-3-C12-IGFR	QHSCRTFYDCIRVLMDDGQLG	6.2	6.4	2.2	2.9	0.3
20E2B-4-G12-IGFR	LDSRRGFYDWIKALIGDRDVQ	9.6	10.0	3.8	2.6	0.4
20E2B-4-G3-IGFR	CQQKGDFYAGLVCLLRERASQ	27.2	23.8	9.1	2.6	0.4
20E2B-3-E4-IGFR	GGSQQSFYDVMCMLLQLDPTC	24.9	22.3	8.9	2.5	0.4
20E2B-3-E2-IGFR	VESDVSFYEGLMRLVWWGQGG	18.6	20.2	8.7	2.3	0.4
20E2B-2-B4-IGFR	ERAGDLFYQWFERLVAGHGLE	5.8	2.3	1.0	2.2	0.5
20E2B-3-C5-IGFR	RMPSGSFYQGIYELVTRQGGF	6.3	2.0	0.9	2.2	0.5

FIG. 20-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYXXhXXhhXXXXXX	--	-- --	--	--	--
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLGLGPSG	18.6	18.1	1.1	16.8	0.1
20E2B-3-C6-IGFR	VEGRGLFYDLLRQLLARRQNG	17.9	16.8	1.1	14.8	0.1
20E2B-4-H3-IGFR	KLHNLMFYYGLQRLVWGAGLG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	0.6	13.8	0.1
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
20E2B-4-H12-IGFR	YSCGDGFYSLLSDLLGGQFRC	6.5	9.7	0.8	12.8	0.1
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	LRIANLFYQRLWDLAFGGGG	15.7	16.7	1.5	11.1	0.1
20E2B-4-F8-IGFR	PVGVQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1
20E2B-1-A11-IGFR	RFSTDGFYQYLLALVGGGPVG	15.0	9.5	1.0	9.7	0.1
20E2B-3-D4-IGFR	NSRDGGFYLQLERLLGFPVTG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	VVTPVNFYRALEALVRGQRLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	QPAPDGFYSALMKLIGRGGVS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	PGTDLGFYQALRCVVIQGACD	11.7	4.9	0.6	8.1	0.1
20E2B-4-F10-IGFR	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	QPDHSYFYSLLQELVGSEERL	11.9	14.7	1.9	7.7	0.1
20E2B-3-D11-IGFR	LGVTDGFYAALGYLIHGVGQF	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	CMMQDGFYAGLGCLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	ICTGQGFYQVLCGLLRGTSAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	QGNVLDFYGWIGRLLAKQGSD	10.3	6.2	0.9	7.3	0.1
20E2B-3-E12-IGFR	VATSQGFYSGLSELLQGGGNV	13.9	6.0	0.8	7.3	0.1
20E2B-2-B8-IGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	0.8	7.2	0.1
20E2B-4-G11-IGFR	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	0.6	7.0	0.1
20E2B-3-D6-IGFR	DSVGDNFYQLLESLVGGHGVG	20.7	17.8	2.6	6.9	0.1
20E2B-2-B7-IGFR	LSSDGQFYRALNLLLQGSAGR	18.0	6.1	0.9	6.7	0.1
20E2B-3-C4-IGFR	ASSASGFYELLQRLAGLGLEV	23.4	20.4	3.3	6.2	0.2

FIG. 20-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYXXhXXhhXXXXXX	--	--	--	--	--
20E2Bβ-4-F7-IR	DALNLRFYSYFQHLMEDQVTD	26.8	3.0	24.2	0.1	8.0
20E2Bβ-3-E12-IR	GNSGGSFYRYFQLLLDSDGMS	17.2	1.4	5.5	0.3	4.0
20E2Bβ-4-F3-IR	GDRVPGFYDWIRQLMVDPLEV	25.2	2.0	7.7	0.3	3.9
20E2Bβ-4-F6-IR	SEREDPFYRWIQAMVEGVSEG	25.7	3.8	11.0	0.4	2.9
20E2Bβ-3-D11-IR	GSVACDFYCHMWSLVEQPAGT	14.8	3.6	4.2	0.9	1.2
20E2Bβ-3-E5-IR	VHPSAGFYKGLLALIGDSQLG	24.3	6.9	4.3	1.6	0.6
20E2Bβ-3-C9-IR	FCGGLSFYGCLQELLTWESPT	29.7	24.3	15.0	1.6	0.6
20E2Bβ-3-C7-IR	QSGSGDFYDWLSRLIRGNGDG	1.5	3.1	1.5	2.0	0.5
20E2Bβ-4-H8-IR	LPRQDGFYDALRRLISEGAGG	25.8	26.9	13.2	2.0	0.5
20E2Bβ-4-G7-IR	LQPCSGFYECIERLIGVKLSG	19.9	25.2	1.6	15.8	0.1

FIG. 2N-3

Clone Design	Sequence XXXXXXFYXXhXXhhXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
20E2Bα-3-B6-IR	SGPMVGFYRGLFSLLSPEDLQ	39.7	34.9	31.5	1.1	0.9
20E2Bα-3-D1-IR	LAEPDSFYNWIAQLLEEGFAG	41.6	35.1	31.7	1.1	0.9
20E2Bα-3-A9-IR	FSGCDNFYSCIQSLWLGPGGV	37.3	35.1	32.4	1.1	0.9
20E2Bα-3-C4-IR	QVFCDNFYHCIETLLGVGQTP	39.6	36.3	33.4	1.1	0.9
20E2Bα-4-F3-IR	RGRDNQFYHGLWALLLGSGLE	37.5	36.6	33.6	1.1	0.9
20E2Bα-4-F4-IR	VSGRGGFYDAIRDLIGPRDQG	37.2	36.9	33.7	1.1	0.9
20E2Bα-3-D4-IR	PVVLDDFYVALCQLMVQGDCF	42.1	38.0	34.5	1.1	0.9
20E2Bα-4-E4-IR	PDIADPFYAFFQGLLRADTPI	40.6	38.4	35.5	1.1	0.9
20E2Bα-4-G10-IR	VAQCTDFYACIRSLVRSGSPG	32.9	31.3	27.1	1.2	0.9
20E2Bα-3-D11-IR	CSQLVSFYLGMDCLLGRGGTQ	34.0	32.5	27.9	1.2	0.9
20E2Bα-3-C8-IR	PLACADFYQCLSDLIRGGPAW	39.2	33.0	28.2	1.2	0.9
20E2Bα-4-F2-IR	VVICTGFYDCIYQLVGSHEEM	38.7	37.6	32.3	1.2	0.9
20E2Bα-4-H12-IR	CVDRRTFYEGLQCLLGGATGD	32.3	30.4	25.8	1.2	0.8
20E2Bα-4-E1-IR	VNLRDPFYQWIEALMDSAGGE	39.2	40.2	32.3	1.2	0.8
20E2Bα-4-H8-IR	LTSSTSFYDALFCLAGLQLCG	37.6	34.8	27.0	1.3	0.8
20E2Bα-3-B4-IR	DFDSSPFYRGLRQLLESRSFP	39.9	34.9	25.9	1.3	0.7
20E2Bα-4-E2-IR	HEAGWTFYDAIQCLVGGWCSK	38.8	36.3	23.5	1.5	0.6
20E2Bα-4-H1-IR	CQQWRSFYHAVSCLLGPDDPD	40.8	33.6	20.2	1.7	0.6
20E2Bα-3-A10-IR	MVDRDPFYQGLRDLIGRQEKG	32.8	32.6	18.5	1.8	0.6
20E2Bα-3-D3-IR	LGRRGGFYRGLQDLIGTQWPR	41.9	29.5	5.6	5.3	0.2

FIG. 2N-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYXXhXXhhXXXXXX	--	--	--	--	--
Parental	FYDAIDQLVRGSARAGGTRD	30.6	15.1	4.2	3.6	0.3
20E2Bα-3-B3-IR	AGVNAGFYRYFSTLLDWWDQG	33.5	1.2	23.5	0.1	20.0
20E2Bα-4-F12-IR	SVKEVQFYRYFYDLLQSEESG	35.5	5.9	27.8	0.2	4.7
20E2Bα-3-B8-IR	IEVTQPFYDYFQQLLRLYGND	39.3	18.2	36.5	0.5	2.0
20E2Bα-3-D2-IR	VQCRADFYSYFACLVGRPGSR	42.6	19.7	26.7	0.7	1.4
20E2Bα-3-A5-IR	RNYPIGFYQFFHELVISSGGG	36.9	22.7	24.5	0.9	1.1
20E2Bα-3-A3-IR	DLGGNSFYYGLLRLVLQDAVG	39.9	33.5	35.5	0.9	1.1
20E2Bα-4-E9-IR	CKDQPDFYMGIKCLISGGGSV	32.8	29.6	28.6	1.0	1.0
20E2Bα-4-G8-IR	ACEGGSFYGCLQSLMSVESGN	37.5	30.5	30.9	1.0	1.0
20E2Bα-4-F9-IR	AVHEDGFYDMLRKLLSEGDSS	35.6	32.5	31.1	1.0	1.0
20E2Bα-4-E7-IR	LARNDEFYRYFEQLVFGDTEG	36.0	31.6	31.2	1.0	1.0
20E2Bα-3-D5-IR	ATCASSFYAQLNCLLSDFDVM	39.5	33.1	31.8	1.0	1.0
20E2Bα-4-F7-IR	VQACQNFYDCLNTLLLLDLGG	36.6	32.9	32.5	1.0	1.0
20E2Bα-3-B12-IR	IRGADQFYQFFRELLEGSVGE	37.0	33.4	33.5	1.0	1.0
20E2Bα-3-A11-IR	RAGSRGFYEFFENLLRVGAGG	36.9	34.9	34.2	1.0	1.0
20E2Bα-3-B7-IR	AQRCADFYACIEELLAPGSWR	40.4	37.1	36.3	1.0	1.0
20E2Bα-3-B5-IR	PGGGEGFYQGLQRLILGADGG	41.6	36.4	34.5	1.1	1.0
20E2Bα-4-G1-IR	QKRSEAFYDWIADLLGQETSG	38.5	28.9	26.5	1.1	0.9
20E2Bα-4-G11-IR	WGLRDDFYRGIRCLVQWSEGC	33.2	30.1	27.8	1.1	0.9
20E2Bα-4-E10-IR	DSTVCGFYCRLAQLVAEGGSP	35.4	30.5	28.0	1.1	0.9
20E2Bα-4-F11-IR	QHSCRTFYDCIRVLMDDGQLG	32.5	29.5	28.0	1.1	0.9
20E2Bα-4-H11-IR	WSGNVDFYYMIRQLCGDVCGS	34.8	32.0	28.7	1.1	0.9
20E2Bα-4-H3-IR	QTVHRDFYAALQDLLINDLGF	38.7	34.9	30.5	1.1	0.9
20E2Bα-4-H7-IR	SSGCQDFYSCMIQLVTGGGGD	35.3	32.5	30.5	1.1	0.9

FIG. 2N-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXX	--	--	--	--	--
20E2A-3-C1-IGFR	GQSPLSFYDAIDQLVRAFPVG	23.4	22.4	20.5	1.1	0.9
20E2A-3-B3-IGFR	AGQLGGFYIAICQLVGYEYCT	21.0	17.0	14.8	1.1	0.9
20E2A-3-D8-IGFR	SAGPLSFYDAIAQLVGPAWRL	22.0	19.7	19.6	1.0	1.0

FIG. 2M-4

Clone Design	Sequence XXXXXXFYDAIDQLVXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
20E2A-4-G2-IGFR	IGVLGSFYDAIDQLVRQGGNR	22.3	17.5	9.9	1.8	0.6
20E2A-4-G3-IGFR	RDVADGFYAAIEQLVRGQFGL	21.2	12.3	6.9	1.8	0.6
20E2A-3-B5-IGFR	VRQAKSFYDAIDQLVRGALRG	24.0	22.7	13.3	1.7	0.6
20E2A-4-H4-IGFR	QVFRGSFYDAIDALVRWGGRA	22.2	20.6	12.0	1.7	0.6
20E2A-4-F8-IGFR	VGAAFSFYDAIDQLVGWSPGS	17.3	17.9	10.7	1.7	0.6
20E2A-3-A6-IGFR	PSPVWSFYDAIQQLVRSGQRG	23.8	23.7	15.0	1.6	0.6
20E2A-4-H12-IGFR	PVSATSFYDAINQLVRMGSRG	25.1	23.5	14.2	1.6	0.6
20E2A-3-B12-IGFR	VMRRDRFYDAIEQLVGGRIGV	27.6	21.9	13.6	1.6	0.6
20E2A-3-B8-IGFR	TTYVNSFYDALQQLLGGDADV	21.5	19.0	12.2	1.6	0.6
20E2A-3-C8-IGFR	LSNMITFYDAINQLVGHVQSL	23.2	17.7	11.4	1.6	0.6
20E2A-4-H10-IGFR	ASSRLSFYDAIEQLIKWSPGP	25.3	23.8	16.2	1.5	0.7
20E2A-3-C9-IGFR	WDLVDSFYDAIDQLVGQRVPG	25.4	21.8	14.6	1.5	0.7
20E2A-4-H2-IGFR	FAFVGSFYDALAQLVAQGPRS	21.8	20.1	13.0	1.5	0.6
20E2A-3-B6-IGFR	EDQPNSFYDAIRQLVMGRLSP	20.3	18.1	11.8	1.5	0.7
20E2A-4-G9-IGFR	SVGPRSFYDAIDQLVGGAWVG	26.0	16.1	10.8	1.5	0.7
20E2A-4-H6-IGFR	KFRVYTFYDAIDQLVNQGRGR	21.9	19.6	13.9	1.4	0.7
20E2A-4-H9-IGFR	GRGWGSFYEAIDQLVRGLGET	24.9	16.8	11.8	1.4	0.7
20E2A-4-G10-IGFR	FTSFHTFYDAIEQLVGQGGDP	25.3	16.5	12.1	1.4	0.7
20E2A-3-A4-IGFR	AGSVTSFYDAMEQLVATGTSA	16.8	2.5	1.8	1.4	0.7
20E2A-3-A7-IGFR	PRESFSFYDAIHQLVTGRVRS	26.0	24.9	19.3	1.3	0.8
20E2A-4-E12-IGFR	LGRADGFYDAIKQLVGADWGG	23.3	23.1	17.8	1.3	0.8
20E2A-3-D1-IGFR	RSGTWTFYDALELLVQGSGSR	24.0	22.4	17.6	1.3	0.8
20E2A-3-C6-IGFR	PVVLFSFYDAIDQLVRKGLGP	23.7	21.7	17.2	1.3	0.8
20E2A-3-D2-IGFR	GRRAQTFYDALEQLVGGEALG	21.4	15.1	11.4	1.3	0.8
20E2A-4-E8-IGFR	AGPDMSFYDAIDQLVHCCGPF	18.4	13.6	10.4	1.3	0.8
20E2A-4-G6-IGFR	HGEKLSFYDAIAQLVGFDIGH	24.7	21.9	17.7	1.2	0.8
20E2A-4-F5-IGFR	GYTPVDFYDAIRQLVTGGWPG	21.7	21.7	18.2	1.2	0.8
20E2A-3-C2-IGFR	FGGFSSFYDALDQLARGRGSD	22.5	19.6	15.8	1.2	0.8
20E2A-4-H8-IGFR	VGIVRGFYEAIERLVGDTHGQ	24.4	18.5	15.1	1.2	0.8
20E2A-3-A5-IGFR	TPGGFSFYDAIQQLVDVLSDS	22.7	15.6	12.6	1.2	0.8
20E2A-3-C11-IGFR	TNAALTFYDAIEQLVRWGQRD	25.8	24.3	21.2	1.1	0.9

FIG. 2M-3

Clon Design	Sequence XXXXXXFYDAIDQLVXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
20E2A-4-F2-IGFR	TGVFNDFYDALQQLVGFRVRD	19.4	19.0	6.8	2.8	0.4
20E2A-3-D4-IGFR	YGSFETFYDAIDQLVRRGSQP	16.1	11.8	4.2	2.8	0.4
20E2A-3-B10-IGFR	RQLLDSFYEAIDQLVRSESRP	24.0	14.3	5.3	2.7	0.4
20E2A-4-E5-IGFR	WPRGDPFYDAMEKLLSQGGGR	18.1	20.6	7.9	2.6	0.4
20E2A-3-D5-IGFR	PGLIQSFYDAIDQLVRQGRGN	15.1	9.3	3.6	2.6	0.4
20E2A-4-G11-IGFR	MNVFVSFYDAIDQLVCQRIGC	20.7	3.3	1.3	2.6	0.4
20E2A-3-C12-IGFR	LDMIGGFYEAIDQLVSGSLAP	25.9	17.4	7.2	2.4	0.4
20E2A-4-G5-IGFR	RRPCNSFYDAIQQLLVGGPCG	23.6	14.6	6.0	2.4	0.4
20E2A-3-D9-IGFR	FGRRSTFYDLIDQLVGQGRGT	19.8	12.3	5.1	2.4	0.4
20E2A-4-F10-IGFR	LRAPRSFYEAIYQLAQRPSVP	21.4	21.6	9.3	2.3	0.4
20E2A-4-E2-IGFR	VQRFSSFYDALDQLVGHGVWK	22.6	21.3	9.1	2.3	0.4
20E2A-4-E3-IGFR	PSARMGFYDLIDQLVGLVPGS	21.0	21.8	10.1	2.2	0.5
20E2A-3-C4-IGFR	SLQPHDFYDAIHRLVFHGGRF	23.5	17.4	7.8	2.2	0.4
20E2A-3-C5-IGFR	ERHGGSFYDAIAQLLQSDRSR	22.2	17.1	7.7	2.2	0.4
20E2A-4-G12-IGFR	YQPPGSFYDWIRELVAGPRRE	24.3	16.3	7.4	2.2	0.5
20E2A-3-C3-IGFR	FAHASSFYDAIDQLVAKCQSP	11.3	2.7	1.2	2.2	0.5
20E2A-3-B7-IGFR	AQSSSGFYEALYQLVWGRGPG	22.3	22.6	10.8	2.1	0.5
20E2A-4-E10-IGFR	TTSGGSFYDAMYQLVWGDWRR	22.6	19.9	9.4	2.1	0.5
20E2A-4-E6-IGFR	ARGTAGFYAELERLVRGQDHG	23.0	16.6	7.9	2.1	0.5
20E2A-3-D3-IGFR	PRHAINFYDAIHQLVFGPGRQ	20.5	15.7	7.6	2.1	0.5
20E2A-4-G4-IGFR	QSAHWSFYDAIERLVNMDTMP	22.6	14.5	7.0	2.1	0.5
20E2A-4-F1-IGFR	VGVVSSFYDAIDQLVGWDRGS	19.6	19.9	9.8	2.0	0.5
20E2A-4-H7-IGFR	DTLIASFYDAIDQLVRLGRNQ	23.0	17.1	8.7	2.0	0.5
20E2A-3-B9-IGFR	FQGTQGFYDAIERLMRRGERP	26.4	22.1	11.5	1.9	0.5
20E2A-4-E9-IGFR	WADWGSFYDAIEQLVQRGGGV	25.3	20.7	11.1	1.9	0.5
20E2A-3-B1-IGFR	EQLSCGFYDAIHQLVHGGGLG	23.1	17.9	9.5	1.9	0.5
20E2A-4-G1-IGFR	CGQRCSFYDAIDQLVGWLPGA	22.6	17.7	9.3	1.9	0.5
20E2A-4-E7-IGFR	MMRVDGFYEAIDRLVNEGQAT	17.2	8.6	4.6	1.9	0.5
20E2A-3-D11-IGFR	RGQATSFYEAIDQLMGGSGGV	16.1	6.1	3.2	1.9	0.5
20E2A-3-A3-IGFR	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
20E2A-4-G7-IGFR	PEGVQGFYDALAHLVGGSLFG	24.4	21.1	11.5	1.8	0.5

FIG. 2M-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX	--	--	--	--	--
Parental	FYDAIDQLVRGSARAGGTRD	30.6	15.1	4.2	3.6	0.3
20E2A-4-F11-IGFR	QGGSASFYDAIDRLLRMRIGG	21.3	18.8	1.3	14.6	0.1
20E2A-4-F12-IGFR	AQGSEGFYDALAQLVGQLVSG	23.3	23.9	3.1	7.8	0.1
20E2A-3-B4-IGFR	GHPAVSFYDAIDQLLRRRGGG	21.8	16.6	2.4	6.9	0.1
20E2A-4-F4-IGFR	YSDTYSFYDAIVQLVRRGASA	20.7	20.0	3.6	5.5	0.2
20E2A-3-C7-IGFR	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1	0.2
20E2A-3-C10-IGFR	RFVWGSFYDAIDQLVQGRWRG	23.3	21.0	4.2	5.0	0.2
20E2A-3-D6-IGFR	RAVGDSFYEAIQQLVRGGHGV	15.1	11.8	2.4	5.0	0.2
20E2A-4-F6-IGFR	LRSQLSFYEAIDQLVQWKGGA	21.5	19.9	4.3	4.6	0.2
20E2A-3-A8-IGFR	DKFFTSFYDAIDQLVQSVRGV	22.2	13.3	2.9	4.6	0.2
20E2A-4-F9-IGFR	MQSGFSFYDAIDRLVGRLGER	21.2	19.0	4.4	4.4	0.2
20E2A-4-F3-IGFR	VGSSSFYEAIERLVQGLGRH	20.6	19.3	4.6	4.2	0.2
20E2A-3-B2-IGFR	LSWAAGFYEAIDQLVRSGGHR	18.7	14.7	3.8	3.9	0.3
20E2A-4-G8-IGFR	QQVHAGFYEALEELVGFGFLG	20.9	10.8	2.7	3.9	0.3
20E2A-3-D10-IGFR	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	LSVALSFYDALGQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
20E2A-3-A11-IGFR	SGSNLGFYDALRQLVGATDGS	17.8	9.7	2.6	3.7	0.3
20E2A-4-H1-IGFR	PSGFLSFYEAIDQLVHGVRWF	20.8	14.5	4.1	3.5	0.3
20E2A-4-F7-IGFR	AFTPTSFYDAIEQLVQQLSPR	19.5	17.9	5.3	3.4	0.3
20E2A-3-D7-IGFR	VSSLRSFYDALDELVRRPFQQ	22.0	18.3	5.6	3.3	0.3
20E2A-3-A9-IGFR	VSMPQSFYDALKQLVRGISEG	24.7	10.5	3.2	3.3	0.3
20E2A-3-A10-IGFR	IGVSRGFYDAIDKLVRDRGSP	26.3	15.4	4.8	3.2	0.3
20E2A-3-B11-IGFR	GRSLLSFYDLIDQLVQAGNGG	15.8	10.7	3.4	3.2	0.3
20E2A-3-D12-IGFR	GQRAQSFYEALARLVCEGRCT	13.9	9.0	2.8	3.2	0.3
20E2A-4-H11-IGFR	CRFQGSFYDAIDLLVLGVRTC	22.8	17.5	5.7	3.1	0.3
20E2A-4-H5-IGFR	RWAFQSFYDAIDHLVNHREGH	20.1	16.6	5.5	3.0	0.3
20E2A-4-E11-IGFR	LPPSSGFYNAIQQLVCGHRGC	21.0	12.6	4.2	3.0	0.3

FIG. 2M-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX	--	--	--	--	--
20E2A-3-C2-IR	RPQGGTFYDMIKQLVLGSGWG	23.4	20.9	15.4	1.4	0.7
20E2A-3-B1-IR	WSAFADFYDAIQHLVAGEVGA	22.1	21.6	15.6	1.4	0.7
20E2A-3-A8-IR	SDGRDGFYDAIQQLVRSAFGD	12.3	4.8	2.7	1.8	0.6
20E2A-4-G2-IR	IRSVFSFYDAIDQLVGKGGWS	18.9	13.8	7.9	1.8	0.6
20E2A-3-A9-IR	GGVSLTFYEAIEQLVRGGFDA	23.3	20.3	11.3	1.8	0.6
20E2A-3-D3-IR	AAQAFSFYDLINQLVASKPSE	24.4	24.5	13.5	1.8	0.6
20E2A-3-A11-IR	QSGACGFYDAINQLVLGVSIC	13.5	4.6	2.7	1.7	0.6
20E2A-3-B4-IR	GGIVFSFYEAIDQLVRGNGAG	21.4	15.3	8.9	1.7	0.6
20E2A-4-E10-IR	IYTGQGFYDAIEQLVRGGSTP	22.3	19.0	11.3	1.7	0.6
20E2A-3-D2-IR	KSPALSFYDAIEQLVGSQGVR	22.5	19.0	11.2	1.7	0.6
20E2A-4-F1-IR	ISPPWTFYDAIDQLVGGSDGR	14.5	6.2	3.9	1.6	0.6
20E2A-3-D1-IR	GSRFRGFYDAIDQLVRQGGLE	16.5	6.6	4.0	1.6	0.6
20E2A-3-D11-IR	GVAGGTFYDAIEQLVRQFGGS	20.2	14.4	8.9	1.6	0.6
20E2A-3-C3-IR	RPLRWSFYDALDQLVGSAIGG	23.9	22.5	14.2	1.6	0.6
20E2A-3-C12-IR	MQGRGGFYDAIADLVGGHVRG	21.3	23.0	14.4	1.6	0.6
20E2A-3-A2-IR	TSQGLSFYDAINQLVAGGWGG	18.9	11.6	7.5	1.5	0.6
20E2A-3-C7-IR	SGGTVTFYDAINQLVQGRYNG	21.6	15.1	6.9	2.2	0.5
20E2A-3-C10-IR	GGALDPFYDAIYQLVIRGSSG	18.1	18.0	9.1	2.0	0.5
20E2A-3-D9-IR	KQRGVTFYDLLNQLVGGSARG	21.8	21.6	8.4	2.6	0.4
20E2A-3-B5-IR	PRAPRSFYDAIHQLVGRQGPG	24.3	18.1	7.4	2.5	0.4
20E2A-3-A6-IR	PCSDDQFYDALSQLVGIRVCP	17.8	19.1	7.6	2.5	0.4
20E2A-4-G12-IR	SYGYQSFYDAIEELVRGPPAR	9.0	9.3	2.6	3.6	0.3

FIG. 2L-3

Clone Design	Sequence XXXXXXFYDAIDQLVXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2A-3-B3-IR	RSSCQSFYDAIERLVLGGTCG	22.4	18.6	15.9	1.2	0.9
20E2A-4-H10-IR	VSRKFSFYDAIQQLVRGDAGV	24.8	24.5	21.2	1.2	0.9
20E2A-4-F8-IR	FQRTWSFYDAINQLVMEGSGD	4.3	2.2	2.1	1.1	0.9
20E2A-4-H5-IR	RGSATTFYDAINQLVGQDGGW	21.3	18.3	16.5	1.1	0.9
20E2A-4-G1-IR	AQPCVSFYDAIEQLVTGRSCM	21.4	18.3	16.0	1.1	0.9
20E2A-4-F2-IR	GGDGDPFYDWIEQLVRAGSEA	20.1	20.5	18.2	1.1	0.9
20E2A-4-H7-IR	LDLCASFYDAIEQLVGVKFCG	22.6	21.2	18.6	1.1	0.9
20E2A-3-D5-IR	WLACQSFYDAIDQLINGGECN	22.7	21.3	18.9	1.1	0.9
20E2A-3-A1-IR	EVNALSFYDAIDQLVRGGLGG	23.8	21.7	19.9	1.1	0.9
20E2A-4-H9-IR	RLQPRTFYEAIDQLIGGVLEG	24.0	22.5	20.8	1.1	0.9
20E2A-3-D7-IR	SGAHRTFYDAIQELVGMGGSK	24.1	23.5	21.0	1.1	0.9
20E2A-4-E5-IR	NMQSLTFYDAIAQLVLGRSGG	24.1	23.5	20.9	1.1	0.9
20E2A-4-E11-IR	RAVGATFYDQINQLVRKDDGY	22.5	14.6	11.7	1.3	0.8
20E2A-3-A4-IR	SQCRGGFYDAIYQLVTGVNCI	20.2	17.5	13.4	1.3	0.8
20E2A-4-G8-IR	DRLAFSFYDAIDQLVHCCGHG	21.7	18.0	13.8	1.3	0.8
20E2A-3-B7-IR	GNRQRGFYDAIDQLVGGSWWR	21.1	21.4	16.9	1.3	0.8
20E2A-4-G11-IR	GGSVLSFYDAIAQLVGGGQSI	22.9	23.1	17.7	1.3	0.8
20E2A-3-C5-IR	RSGPMSFYDAIEQLVLGRLHP	24.2	24.3	19.0	1.3	0.8
20E2A-4-E8-IR	VSGCRTFYDAIDQLVSGQACG	17.1	11.5	9.4	1.2	0.8
20E2A-4-H2-IR	AQFPRTFYDAIEQLIHGKGMD	21.6	13.7	11.6	1.2	0.8
20E2A-4-H4-IR	CAQPESFYDAIDRLVTGRCLV	21.3	19.6	16.3	1.2	0.8
20E2A-3-D4-IR	PDECQSFYCAIDRLVTGKGGR	23.2	22.2	18.0	1.2	0.8
20E2A-4-F5-IR	QRRARDFYEAIQQLVGGVAGL	12.2	5.7	3.8	1.5	0.7
20E2A-3-B10-IR	PLVRGTFYDAIKQLVMGGSSD	14.9	5.9	3.9	1.5	0.7
20E2A-3-B9-IR	VGIAWTFYDAIQQLVRGSPEG	15.5	11.0	7.2	1.5	0.7
20E2A-3-D10-IR	PRGQASFYDMIEQLVGSADWN	22.2	19.1	12.8	1.5	0.7
20E2A-3-D6-IR	DGRVWSFYDALEQLVGQFEGP	21.8	19.3	13.0	1.5	0.7
20E2A-4-G9-IR	RFVVRSFYDAIEQLILAPNLG	21.3	19.9	13.3	1.5	0.7
20E2A-4-E1-IR	KVGRGSFYDAIRELVGQGGHV	23.1	20.7	13.6	1.5	0.7
20E2A-4-F12-IR	PAIGFTFYDAIRQLVWFQGAD	17.5	17.1	12.1	1.4	0.7
20E2A-4-G3-IR	ALPGRSFYDAIAQLVGPDWGA	21.6	19.4	14.1	1.4	0.7

FIG. 2L-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX	--	--	--	--	--
Parental	FYDAIDQLVRGSARAGGTRD	30.6	15.1	4.2	3.6	0.3
20E2A-4-F9-IR	PPWGARFYDAIEQLVFDNLCC	19.9	1.2	13.9	0.1	11.5
20E2A-4-E2-IR	IGRVRSFYDAIDKLFQSDWER	13.9	1.3	9.7	0.1	7.6
20E2A-3-B6-IR	RDAGSSFYDAIDQLVCLTYFC	14.5	1.6	6.1	0.3	3.8
20E2A-3-A7-IR	MPMGLNFYDGIEQLVREWGGD	18.6	4.2	15.2	0.3	3.6
20E2A-4-F7-IR	TISAHTFYEAIYQLIEGIDPL	20.1	10.6	15.8	0.7	1.5
20E2A-3-C9-IR	SPWGRAFYDALDQLMGGAERG	24.4	16.4	23.8	0.7	1.4
20E2A-3-C11-IR	LSPPRDFYDAIQQLVRDGGWG	14.8	5.9	6.6	0.9	1.1
20E2A-4-G7-IR	HGVPRTFYDAIDQLVWGIEVG	17.2	8.3	8.9	0.9	1.1
20E2A-4-H11-IR	GGTDQLFYGAIDQLVGGTWWR	25.4	26.9	25.6	1.1	1.0
20E2A-4-E9-IR	LSVHQSFYDAINELIFSGLEA	4.7	2.1	2.1	1.0	1.0
20E2A-4-F4-IR	GDARDPFYDAMEQLVYGELGG	12.6	5.6	5.7	1.0	1.0
20E2A-3-A3-IR	VASPRSFYEAIAQLVFNLGQE	21.0	11.9	12.1	1.0	1.0
20E2A-4-E4-IR	RKPCQTFYDCILDLVVTDVDV	21.0	16.0	16.1	1.0	1.0
20E2A-4-G5-IR	LLSRWTFYDAIEQLVGGGADG	22.1	19.5	18.8	1.0	1.0
20E2A-4-H1-IR	PAGCQGFYEAIEQLVTGGECG	24.4	20.4	21.2	1.0	1.0
20E2A-3-D8-IR	AVFPRTFYEAIDQLVGVSLLG	22.3	20.8	20.3	1.0	1.0
20E2A-4-F11-IR	APIPFSFYDAIVQLVMQGDHE	23.2	21.0	20.4	1.0	1.0
20E2A-3-C1-IR	QCNPRTFYEAIAQLVTGCDVS	23.4	22.3	22.5	1.0	1.0
20E2A-3-B2-IR	VSTSGSFYDAIQQLLEDSWGW	24.6	22.5	22.8	1.0	1.0
20E2A-4-G10-IR	HHSAFSFYDAIAQLVGVPWEE	21.5	22.9	22.2	1.0	1.0
20E2A-3-A5-IR	FTYVHSFYDAIEQLVRGEGGG	24.6	23.4	22.6	1.0	1.0
20E2A-4-H3-IR	QGNAQNFYDAIDQLCFGCLGG	24.5	24.1	25.1	1.0	1.0
20E2A-3-C4-IR	SSEGWTFYDAIDQLVGRERGW	25.2	24.4	24.7	1.0	1.0
20E2A-4-E3-IR	PDGCATFYHAIQQLVTGFPCV	18.5	15.7	13.6	1.2	0.9
20E2A-4-E7-IR	RGPPMTFYDAIAQLVAQSADG	17.8	16.6	14.4	1.2	0.9

FIG. 2L-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	EHWNTVDPFYHKLSELLRESGA	--	--	--	--	--
B6C-3-C4-IGFR	EHWNTVDPFYLKMTELLRESGA	21.0	23.1	--	--	--
B6C-3-F5-IGFR	EHWNTVDPFYHKLEELLRESGA	26.5	22.6	--	--	--
B6C-3-D4-IGFR	EHWNTVDPFYHKLYELLRESGA	26.6	22.1	--	--	--
B6C-3-A3-IGFR	EHWNTVDPFYQKLSELLRESGA	29.7	20.8	--	--	--
B6C-3-C9-IGFR	EHW?TVDPFYLKLSELL?ESGA	29.6	20.5	--	--	--
B6C-3-C8-IGFR	EHWNTVDPFYQRLFELLRESGA	30.5	19.9	--	--	--
B6C-3-A8-IGFR	EHWNTVDPFYHGLQELLRESGA	29.7	19.7	--	--	--
B6C-3-A2-IGFR	EHWNTVDPFYHRLSELLRESGA	33.6	19.0	--	--	--
B6C-3-A9-IGFR	EHWNTVDPFYHNLYELLRESGA	33.0	18.3	--	--	--
B6C-3-C10-IGFR	EHWNTVDPFYNKLSELLRESGA	30.0	17.7	--	--	--
B6C-3-F7-IGFR	EHWNTVDPFYQKLSELLRESSA	27.3	17.6	--	--	--
B6C-3-F10-IGFR	EHWNTVDPFYAKLSALLRESGA	27.4	17.4	--	--	--
B6C-3-C12-IGFR	EHWNTVDPFYARLSALLRESGA	28.4	17.3	--	--	--
B6C-3-E11-IGFR	EHWNTVDPFYHILAELLRESGA	28.0	17.2	--	--	--
B6C-3-F8-IGFR	EHWNTVEP*YHKLCGRLRESGA	25.4	16.0	--	--	--
B6C-3-F2-IGFR	EHWNTVDPFYDRLTELLRESGA	30.8	15.1	--	--	--
B6C-3-B6-IGFR	EHWNTVDPF*PKVSELLRESGA	2.9	14.7	--	--	--
B6C-3-D5-IGFR	EHWNTVEPFGA*LAEPLRESGA	2.4	14.3	--	--	--
B6C-3-A4-IGFR	ERWNTVDPFYHKLSELLRESGA	22.6	13.9	--	--	--
B6C-3-D3-IGFR	EHWNTVDQFYQALFELLRESGA	28.0	13.8	--	--	--
B6C-3-F9-IGFR	EHWNTVDPFYHKLAELLRESGA	24.8	13.6	--	--	--
B6C-3-A7-IGFR	EHWNTVDPFYDKLSDLLRESGA	14.4	12.8	--	--	--
B6C-3-H10-IGFR	EYWNTVDPFYHKLAELLRESGA	17.1	11.1	--	--	--
B6C-3-H11-IGFR	EHWNTVRPRYQ*LSELLRESGA	2.3	11.0	--	--	--
B6C-3-A1E-IGFR	EHWNTVDPFYHKLQELLRESGA	23.3	10.5	--	--	--
B6C-3-E12-IGFR	EHWNTVATF*DKVTDLLRESGA	2.7	10.1	--	--	--
B6C-3-H12-IGFR	EHWNTVDPFLDKRS*LLRESGA	2.3	9.9	--	--	--
B6C-3-G1-IGFR	EHWNTVGAFQPTPEYLLRESGA	--	--	--	--	--

FIG. 2K

Clone Design	Sequence EHWNTVDPFYHKLSELLRESG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
B6C-4-F4-IR	EHWNTVDPFYQKLFELLRESG	36.3	36.9	34.3	1.1	0.9
B6C-3-D2-IR	EHWNTVDPFYH*LAELLRESG	8.5	10.3	9.0	1.2	0.9
B6C-3-A1-IR	EHWNTVDPFYH*LNELLRESG	26.5	30.7	16.8	1.8	0.5
B6C-3-B5-IR	EHWNTVDPFYHKLQELLRESG	33.4	33.0	15.0	2.2	0.5
B6C-3-A4-IR	EHWNTVDPFYRRLQELLRESG	33.6	31.8	13.5	2.4	0.4

FIG. 2J-3

Clone Design	Sequence EHWNTVDPFYHKLSELLRESG	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
B6C-3-C11-IR	EHWNTVDPFYD*ISELLRESG	33.4	35.0	36.7	1.0	1.0
B6C-3-C4-IR	EHWNTVDPFYHLLQELLRESG	34.5	35.2	36.8	1.0	1.0
B6C-3-B6-IR	EHWNTVDPFYHMLQELLRESG	34.2	36.1	36.9	1.0	1.0
B6C-4-E5-IR	EHWNTVDPFYH*MSDLLRESG	35.5	35.8	36.9	1.0	1.0
B6C-4-H12-IR	EHWNTVDPFYHYLQDLLRESG	37.0	36.2	37.1	1.0	1.0
B6C-4-F9-IR	EHWNTVDSFYLGLQELLRESG	36.1	36.2	37.3	1.0	1.0
B6C-3-A6-IR	EHWNTVDPFYQGLSELLRESG	35.8	38.0	37.4	1.0	1.0
B6C-3-D1-IR	EHWNTVDPFYQALQQLLRESG	36.2	37.8	37.5	1.0	1.0
B6C-3-D4-IR	EHWNTVDPFYLMLQELLRESG	35.9	36.4	37.5	1.0	1.0
B6C-3-C1-IR	EHWNTVDPFYLKMQDLLRESG	36.8	36.6	37.9	1.0	1.0
B6C-4-G5-IR	EHWNTVDPFYQKLQELLRESG	36.5	37.9	38.0	1.0	1.0
B6C-3-A8-IR	EHWNTIDPFYHQISELLRESG	34.4	37.0	38.1	1.0	1.0
B6C-4-H1-IR	EHWNTVDPFYH*MTELLRESG	36.8	36.5	38.3	1.0	1.0
B6C-3-D10-IR	EHWNTVDPFYHYMSQLLRESG	37.0	37.0	38.5	1.0	1.0
B6C-3-D12-IR	EHWNTVDPFYQGLFELLRESG	36.2	37.0	38.7	1.0	1.0
B6C-3-B9-IR	EHWNTVDPFYAKLQELLRESG	36.3	37.3	38.8	1.0	1.0
B6C-4-H7-IR	EHWNTVDPFYH*MRELLRESG	37.5	38.0	39.1	1.0	1.0
B6C-3-D11-IR	EHWNTVDPFYHGL*ELLRESG	36.1	37.5	39.2	1.0	1.0
B6C-4-F10-IR	EHWNTVDPFYLGLQELLRESG	37.9	38.6	39.3	1.0	1.0
B6C-4-G8-IR	EHWNTVDPFYD*IADLLRESG	35.9	38.0	39.7	1.0	1.0
B6C-3-A9-IR	EH*NTVDPFYHGLYELLRESG	36.5	38.8	39.9	1.0	1.0
B6C-3-A7-IR	EHWNTVDAFYHGLQELLRESG	38.1	39.4	40.2	1.0	1.0
B6C-4-F12-IR	EHWNTVDPFYQGLIELLRESG	38.0	38.4	40.2	1.0	1.0
B6C-4-G9-IR	EHWNTVDPFYN*MRELLRESG	37.5	39.4	40.5	1.0	1.0
B6C-4-H8-IR	EHWNTVDPFYQGLLDLLRESG	38.5	40.0	40.8	1.0	1.0
B6C-3-B10-IR	EHWNTVDPFYQKLQDLLRESG	39.3	40.3	40.9	1.0	1.0
B6C-3-A10-IR	EHWNTVDPFYHGLQELLRESG	38.4	40.9	41.6	1.0	1.0
B6C-3-A3-IR	EHWNTVDPFYH*MSELLRESG	39.2	40.0	41.7	1.0	1.0
B6C-3-A5-IR	EHWNTVDPFYAGLQALLRESG	38.2	40.4	41.9	1.0	1.0
B6C-3-C3-IR	EHWNTVDPFYHMLQKLLRESG	34.5	34.6	32.0	1.1	0.9

FIG. 2J-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	EHWNTVDPFYHKLSELLRESG	--	--	--	--	--
B6C-3-C7-IR	EHWNTVDPFYFTLFE*LRESG	31.7	2.1	20.0	0.1	9.4
B6C-4-F2-IR	EHWNTVDPFYNQLWEWLRESG	8.2	1.8	4.3	0.4	2.4
B6C-3-A2-IR	EHWNTVDPFYHQLSEWLRESG	34.9	18.1	36.0	0.5	2.0
B6C-4-H11-IR	EHWNTVDPFYQQLYEWLRESG	37.1	28.2	38.6	0.7	1.4
B6C-4-H4-IR	EHWNTVDPFYRQLSEWLRESG	39.5	28.3	39.4	0.7	1.4
B6C-3-A11-IR	EHWNTVDPFYHYFQELLRESG	25.4	25.9	34.2	0.8	1.3
B6C-3-D9-IR	EHWNTVDPFYHQMYEWLRESG	35.7	30.3	37.2	0.8	1.2
B6C-4-G4-IR	EHWNTVDPFYRQLYEWLRESG	35.3	31.0	38.4	0.8	1.2
B6C-3-C6-IR	EHWNTVDLFYYGLQELLRESG	33.3	33.9	35.9	0.9	1.1
B6C-3-D8-IR	EHWNTVDPFYH*ISELLRESG	34.5	34.7	37.1	0.9	1.1
B6C-4-G7-IR	EHWNTVDPFYQFFAELLRESG	35.9	36.9	38.9	0.9	1.1
B6C-3-C8-IR	EH*NTVDPFYEGLLELLRESG	35.6	37.2	39.6	0.9	1.1
B6C-3-D6-IR	EH*NTVDPFYQGLFELLRESG	37.6	37.6	40.2	0.9	1.1
B6C-3-C10-IR	EHWNTVDPFYQYFSELLRESG	35.3	36.4	40.6	0.9	1.1
B6C-3-B3-IR	EHWNTVDPFYYGLQTLLRESG	38.3	38.7	40.8	0.9	1.1
B6C-3-B1-IR	EHWNTVDPFYQALFELLRESG	37.8	38.9	41.2	0.9	1.1
B6C-4-F6-IR	EHWNTVDPFYD*MRNLLRESG	35.8	36.8	38.7	1.0	1.1
B6C-3-B11-IR	EHWNTVDPFYNLLQELLRESG	36.3	37.0	38.8	1.0	1.1
B6C-3-B8-IR	EHWNTVDPFYDGLRQLLRESG	37.2	39.2	41.2	1.0	1.1
B6C-3-C12-IR	EHWNTVDPFYGKLQELLRESG	28.3	28.7	28.9	1.0	1.0
B6C-3-C2-IR	EHWNTVDPFYQQLFELLRESG	34.1	34.7	33.8	1.0	1.0
B6C-3-D5-IR	EHWNTVDPFYLMLQQLLRESG	33.9	35.3	34.1	1.0	1.0
B6C-4-F7-IR	EH*NTVDPFYHKLYELLRESG	34.9	34.7	34.2	1.0	1.0
B6C-4-H2-IR	EHWNTVDPFYH*MSNLLRESG	35.4	35.8	35.8	1.0	1.0
B6C-3-B12-IR	EHWNTVDPFYY*MSELLRESG	33.6	35.2	36.0	1.0	1.0
B6C-3-A12-IR	EHWNTVDPFYQLLFELLRESG	33.1	37.0	36.2	1.0	1.0
B6C-4-E9-IR	EHWNTVDPFYQRMFELLRESG	36.1	36.0	36.2	1.0	1.0
B6C-4-E8-IR	EHWNTVDPFYQGLWELLRESG	34.2	35.0	36.6	1.0	1.0

FIG. 2J-1

Clon	Sequence	Ratios over Background			Comparisons	
Design	OOUUUUUUJJJJDPFYHKLSELXXOO	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	-- --	--	--	--
B6H-4-D8-IGFR	GGVKAAAAERDDSDPFYHKLSELLFGS	15.1	16.4	--	--	--
B6H-4-D6-IGFR	CCEMVKTIEHGGNDPFYHKLSELVFGR	12.6	15.6	--	--	--
B6H-4-E10-IGFR	GGAKVAVVVDHGDDPFYHKLSELLRGS	10.2	15.1	--	--	--
B6H-4-F5-IGFR	RGKTKMAMAAGGNRDPFYHKLSELIFGN	12.3	14.8	--	--	--
B6H-4-B2-IGFR	GGMATKIVTAPGHDPFYHKLSELLFGG	6.6	11.8	--	--	--
B6H-3-F3-IGFR	SGEGEMAMPGPDDPFYHKLSELIGSRA	8.2	11.6	--	--	--
B6H-3-A2-IGFR	GGMAEVVVVGPPRDPFYHKLSELVGGG	10.9	9.9	--	--	--
B6H-3-H2-IGFR	GGEVKVMVADGSTDPFYHKLSELLGRT	5.9	9.6	--	--	--
B6H-4-A1-IGFR	SCVMVETVAGRNRDPFYHKLSELVGGC	4.4	9.5	--	--	--
B6H-3-H1-IGFR	RRW*KVPGAADPFYHKLSELLGRSA	7.2	8.7	--	--	--
B6H-4-C2-IGFR	GGVEATEVEHADGDPFYHKLSELVGRS	6.7	8.6	--	--	--
B6H-4-H9-IGFR	RGVEVAVITHGPPDPFYHKLSELLRGA	12.3	8.4	--	--	--
B6H-4-B7-IGFR	SGTVTVIAMSGTDDPFYHKLSELLSRS	6.4	8.2	--	--	--
B6H-4-A7-IGFR	GRTAVVKEASPAHDPFYHKLSELLLRG	9.7	8.1	--	--	--
B6H-4-B3-IGFR	RGAIGNAAVGNRSDPFYHKLSELISRG	4.4	7.8	--	--	--
B6H-4-B4-IGFR	GGMIKTAMEHDTRDPFYHKLSELLRGG	5.2	7.4	--	--	--
B6H-4-E1-IGFR	GCAEVEEVAGAGHDPFYHKLSELCAGG	3.6	7.1	--	--	--
B6H-3-C1-IGFR	SSVVVVEVVDARRDPFYHKLSELV?SG	5.7	4.6	--	--	--
B6H-4-A3-IGFR	GRKKAVATMTDGGDPFYHKLSELILRS	4.4	4.2	--	--	--
B6H-4-H10-IGFR	RGETEMAVADTDDDPFYHKLSELLGRG	4.4	3.2	--	--	--
B6H-3-G1-IGFR	GQRDPFYHKLSELMGRGA	2.4	2.9	--	--	--

FIG. 21-2

Clone Design	Sequence OOUUUUUUJJJJDPFYHKLSELKXOO	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	-- --	--	--	--
B6H-3-F1-IGFR	RRVAAVA?KDATGDPFYHKLSELLRSG	20.0	30.8	--	--	--
B6H-3-D4-IGFR	RSTMKEKIEGDGNDPFYHKLSELLKSG	19.0	27.6	--	--	--
B6H-3-G3-IGFR	GGAVIVTAARRGSDPFYHKLSELVGRG	14.2	25.2	--	--	--
B6H-3-F2-IGFR	SREAVEVTMARGSDPFYHKLSELVWGS	12.5	24.8	--	--	--
B6H-3-D1-IGFR	RSTTMVKAVPPPRDPFYHKLSELL*GG	20.0	24.2	--	--	--
B6H-3-A3-IGFR	GRTEVVVVGTRRDPFYHKLSELLASG	14.2	22.8	--	--	--
B6H-3-A4-IGFR	RRMAGWQ*TSSSDPFYHKLSELVSGS	13.0	22.8	--	--	--
B6H-3-B4-IGFR	SRKEVTEMVGGPSDPFYHKLSELMGSG	10.2	22.8	--	--	--
B6H-4-D11-IGFR	RGTAKQRKSSDP*DPFYHKLSELIYGS	14.0	22.5	--	--	--
B6H-3-G2-IGFR	GGVVAVVAAGRRDDPFYHKLSELVSGR	15.2	22.5	--	--	--
B6H-3-B2-IGFR	SR.MAMVEVGNPGDPFYHKLSELLGS	14.5	21.9	--	--	--
B6H-3-E2-IGFR	RRVTAVIEVDGADDPFYHKL?ELLSGG	11.6	21.8	--	--	--
B6H-3-B1-IGFR	RSVIAN??G?NADPFYHKLSELISSG	15.9	21.7	--	--	--
B6H-4-G3-IGFR	RGVVIETTKDPGADPFYHKLSELLFGR	19.1	21.4	--	--	--
B6H-4-F9-IGFR	RRTTVMETVGGRDDDPFYHKLSELLHRG	11.3	20.9	--	--	--
B6H-3-E3-IGFR	GRVVVAAAVRPDDDPFYHKLSELVAGR	14.2	20.8	--	--	--
B6H-3-E1-IGFR	RGVATVVVANHHSDPFYHKLSELVLRG	20.0	20.6	--	--	--
B6H-4-F3-IGFR	RRKMATEIMRSDADPFYHKLSELLGGS	12.5	20.3	--	--	--
B6H-3-D2-IGFR	GGKTAVEVTSPASDPFYHKLSELLLRG	12.1	19.3	--	--	--
B6H-4-A9-IGFR	RREKKVKVTTTDNDPFYHKLSELVFGG	14.1	19.2	--	--	--
B6H-4-E6-IGFR	SSAIIMVAADRADDPFYHKLSELLWGS	12.5	19.2	--	--	--
B6H-4-C3-IGFR	RREVAIVAAGAGGDPFYHKLSELLSRG	23.6	18.9	--	--	--
B6H-3-C2-IGFR	RRMVMEAAENHADDPFYHKLSELLWRD	16.2	18.5	--	--	--
B6H-3-C4-IGFR	GRKMEIVAIRGAHDPFYHKLSELL*GR	16.8	17.2	--	--	--
B6H-3-C3-IGFR	CCIAMVEMAAGGGDPFYHKLSELLSGR	14.6	17.1	--	--	--
B6H-3-B3-IGFR	RGAQSPDPFYHKLSELAFGS	9.0	16.8	--	--	--
B6H-4-H3-IGFR	RKTAMVVIGDASDPFYHKLSELAFGS	10.1	16.6	--	--	--
B6H-3-H3-IGFR	GSVITKAMKADGDDPFYHKLSELL*GG	14.2	16.4	--	--	--

FIG. 2I–1

Clone Design	Sequence OOUUUUUUUUJJJJDPFYHKLSELXXOO	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
B6Hβ-3-H12-IR	RGEAKEAKIGSAGDPFYHKLSELMQGSR	33.6	32.0	29.5	1.1	0.9
B6Hβ-3-G10-IR	GCEEVVVMANSSADPFYHKLSELCQGSR	30.1	34.3	30.5	1.1	0.9
B6Hβ-3-F10-IR	GCAAVVVTTGGDNDPFYHKLSELLQGCR	37.1	35.3	32.4	1.1	0.9
B6Hβ-3-D5-IR	SRTGERQVVGSHADPFYHKLSELLLSS	39.9	38.9	35.5	1.1	0.9
B6Hβ-3-B12-IR	GCKEVVVETAHADDPFYHKLSELLQGCR	39.5	40.0	37.1	1.1	0.9
B6Hβ-3-D2-IR	RRITIKVKAGDDDDPFYHKLSELLWGG	40.4	41.5	39.1	1.1	0.9
B6Hβ-3-D1-IR	WCDQKETVVSNSDDPFYHKLSELVGCS	41.1	44.6	36.6	1.2	0.8
B6Hβ-3-G6-IR	RCEEITIGDGRAGDPFYHKLSELLQGC	34.3	36.4	24.1	1.5	0.7
B6Hβ-3-A7-IR	CSVVMTEEKNDRDDPFYHKLSELLQGC	38.1	30.9	18.4	1.7	0.6
B6Hβ-3-B10-IR	GGEARRRQQVGTANDPFYHKLSELAFGGR	32.3	36.5	22.8	1.6	0.6
B6Hβ-3-B9-IR	GCAVTAITINGTSDPFYHKLSELCQGS	38.6	38.5	20.8	1.9	0.5
B6Hβ-3-D6-IR	GSKVKAMAVGTSDDPFYHKLSELVQGR	35.9	36.0	15.6	2.3	0.4
B6Hβ-3-C7-IR	RCKGIKAHSDNDPFYHKLSELCQGG	38.3	38.0	6.6	5.8	0.2

FIG. 2H-4

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	OOUUUUUUUJJJJDPFYHKLSELXXOO	--	--	--	--	--
B6Hβ-3-D7-IR	RGVEMKAIVVGTPNDPFYHKLSELSSGS	39.5	27.1	26.3	1.0	1.0
B6Hβ-3-B2-IR	CSAVKMAEAGDPSDPFYHKLSELCQGS	34.3	34.4	35.4	1.0	1.0
B6Hβ-3-G5-IR	RGGDGDPFYHKLFELMQSS	35.3	35.0	35.6	1.0	1.0
B6Hβ-3-H1-IR	WLCKRQTHDPDPFYHKLSELAGCR	36.8	35.4	36.5	1.0	1.0
B6Hβ-3-A5-IR	SSKVVKATVGTPHDPFYHKLSELLQGS	34.9	35.5	35.9	1.0	1.0
B6Hβ-3-H11-IR	GCAAIAVATGNDNDPFYHKLSELLQGCR	37.7	36.4	37.6	1.0	1.0
B6Hβ-3-C2-IR	GCAAVVKETHDPPDPFYHKLSELLHGC	37.4	36.5	37.2	1.0	1.0
B6Hβ-3-C8-IR	SCAAEKEVAGTARDPFYHKLSELMQSS	37.0	37.7	39.5	1.0	1.0
B6Hβ-3-A11-IR	CSVAVGDSGDPFYHKLSELLQGCR	40.4	38.2	39.1	1.0	1.0
B6Hβ-3-D8-IR	WQRNKQQIIGTPDDPFYHKLSELLEGS	35.4	38.3	39.5	1.0	1.0
B6Hβ-3-B7-IR	RSAAAKAVIGSPNDPFYHKLSELIQGG	37.8	39.0	39.4	1.0	1.0
B6Hβ-3-A12-IR	WLCDRDGRDEQPWDPFYHKLSELVSCGR	33.5	39.4	41.3	1.0	1.0
B6Hβ-3-B4-IR	GSVAAAKKTGSSDDPFYHKLFELLQGS	39.0	39.8	41.1	1.0	1.0
B6Hβ-3-A4-IR	GCAVTTMTMRSPADPFYHKLSELCQGR	40.1	40.4	41.1	1.0	1.0
B6Hβ-3-E12-IR	GCKVDDE*ARSSDPFYHKLSELLKGCR	35.8	40.7	40.7	1.0	1.0
B6Hβ-3-B8-IR	GCKAVVEVKDHGDDPFYHKLSELLQGC	40.8	40.7	39.5	1.0	1.0
B6Hβ-3-C5-IR	CSTVTVSGSDDPFYHKLSELLQGC	40.7	40.9	42.6	1.0	1.0
B6Hβ-3-A2-IR	RSVTAKVEVGSDRDPFYHKLSELLQGS	41.1	41.4	41.9	1.0	1.0
B6Hβ-3-A8-IR	GSRRQKIEVGTPNDPFYHKLSELLQGG	40.0	41.9	41.6	1.0	1.0
B6Hβ-3-C12-IR	LCDEKQRVTGGTNDPFYHKLSELTGGCR	39.8	42.0	41.3	1.0	1.0
B6Hβ-3-B11-IR	SCMVEGPNDDPFYHKLSELLQGCR	40.7	42.6	43.3	1.0	1.0
B6Hβ-3-C3-IR	GGAAVVVAMGGNDDPFYHKLFELMQGG	43.0	42.7	44.0	1.0	1.0
B6Hβ-3-B5-IR	GGVIKAMKAGGPDDPFYHKLSELLQGS	42.7	43.9	45.4	1.0	1.0
B6Hβ-3-C4-IR	GCIIAEKVVGPPDDPFYHKLSELLDCG	41.1	42.3	36.6	1.2	0.9
B6Hβ-3-G7-IR	GCEKVVAVAGNAGDPFYHKLSELLQGC	4.1	2.4	2.1	1.1	0.9
B6Hβ-3-C11-IR	GSVMTVTEMAGADDPFYHKLSELLQGGR	29.2	30.6	28.2	1.1	0.9

FIG. 2H-3

Clone Design	Sequence OOUUUUUUUJJJJDPFYHKLSELXXOO	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
B6Hα-1-A6-IR	GCKMEETETGTSDDPFYHKLSELCSGG	49.7	34.5	4.0	8.6	0.1
B6Hα-2-C5-IR	RGEVATMEVPAGGDPFYHKLSELLWGS	42.6	34.2	3.3	10.4	0.1
B6Hα-2-C4-IR	RCGRW*AEMGAGDDPFYHKLSELVCG	20.7	9.9	0.9	11.0	0.1
B6Hα-2-C9-IR	RCMVETIAVGSGDDPFYHKLSELCQGG	47.4	32.6	2.8	11.6	0.1
B6Hα-4-H8-IR	WWQKKSGDGASASDPFYHKLSELIWGS	36.3	28.1	2.4	11.7	0.1
B6Hα-3-F11-IR	RGMKEEVLVGGSTDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1
B6Hα-3-E9-IR	RCEEKQAEVGPSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
B6Hα-1-A2-IR	RGCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
B6Hα-1-B5-IR	CCTTEMVVMDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
B6Hβ-3-G4-IR	GCKKVEAKKGNDADPFYHKLSELLQGC	36.4	28.4	36.0	0.8	1.3
B6Hβ-3-A10-IR	RSMMAKAIVGGPGDPFYHKLYELQFGSR	36.7	27.9	34.7	0.8	1.2
B6Hβ-3-D9-IR	CGGAVPDGDDPFYHKLSELMQGC	34.9	32.1	35.6	0.9	1.1
B6Hβ-3-A3-IR	GCEEVEAETTGHRDPFYHKLSELLQGC	36.3	33.7	37.3	0.9	1.1
B6Hβ-3-G1-IR	GCAEIEIAAGGGGDPFYHKLSELLQGC	34.7	33.7	35.9	0.9	1.1
B6Hβ-3-B3-IR	GCAEVKAVKGAGDDPFYHKLSELLQGC	35.9	35.1	37.4	0.9	1.1
B6Hβ-3-G11-IR	GCAAVETTTNGRNDPFYHKLSELLQGCR	37.6	36.2	39.0	0.9	1.1
B6Hβ-3-F5-IR	CGEVTGRAGDPFYHKLSELLQGC	39.2	37.2	41.0	0.9	1.1
B6Hβ-3-A1-IR	GCAMVEATEGRRHDPFYHKLSELIQGC	41.3	38.0	43.0	0.9	1.1
B6Hβ-3-H3-IR	GCTEVVGSGDDPFYHKLSELLQGC	39.0	38.3	40.7	0.9	1.1
B6Hβ-3-D3-IR	GQCAMEEIIRGANDPFYHKLSELCEGG	38.8	38.4	41.3	0.9	1.1
B6Hβ-3-C9-IR	GCAEIVIEEGDDSDPFYHKLSELLQGC	36.7	39.2	41.5	0.9	1.1
B6Hβ-3-F1-IR	PQCSSIKAEGGSDDPFYHKLSELLVGC	41.5	40.0	42.2	0.9	1.1
B6Hβ-3-C6-IR	GCAAVVAEASGDDPFYHKLSELLQGC	39.9	40.3	42.7	0.9	1.1

FIG. 2H-2

Clone	Sequence	Ratios over Background			Comparis ns	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	OOUUUUUUUJJJJDPFYHKLSELXXOO	--	--	--	--	--
B6Hα-3-F5-IR	GGAVAAAVVGSRADPFYHKLSELVQGS	42.7	9.6	17.9	0.5	1.9
B6Hα-2-D10-IR	SGGGQQRKAIATSDDPFYHKLSELLLGG	20.8	1.7	1.1	1.5	0.6
B6Hα-3-F1-IR	CSMAAVAEAGDDDDPFYHKLSELCQGS	22.5	2.4	1.3	1.8	0.5
B6Hα-3-E6-IR	CGAKMTGTPNDPFYHKLSELLQRG	18.2	2.3	1.2	1.9	0.5
B6Hα-1-B8-IR	CCVEAEEAVGRRGDPFYHKLSELTGCC	44.6	5.2	2.1	2.5	0.4
B6Hα-2-D5-IR	SRVVTMVIKRGSPDPFYHKLSELVQGR	39.6	2.3	0.9	2.6	0.4
B6Hα-1-B3-IR	GCITAENGAGDPFYHKLSELGGCS	33.1	3.2	1.1	2.9	0.3
B6Hα-3-E5-IR	RCGDEEGWQENRRDDPFYHKLSELFGGC	28.8	2.9	1.0	2.9	0.3
B6Hα-4-H9-IR	GCEVIAAEGRRDDPFYHKLSELCQGG	17.4	6.4	2.1	3.0	0.3
B6Hα-2-D8-IR	SSETAKMVTGTRDDPFYHKLSELVQGS	19.3	3.0	1.0	3.0	0.3
B6Hα-3-E4-IR	WLCDGGWKQRRPPGDPFYHKLSELIDCG	43.1	8.7	2.8	3.1	0.3
B6Hα-3-F7-IR	SRVAATKEKRPSDDPFYHKLSELLQGS	41.5	3.1	1.0	3.1	0.3
B6Hα-1-A3-IR	SRAKVEAEMPDSGDPFYHKLSELLASG	37.4	2.6	0.8	3.3	0.3
B6Hα-4-H10-IR	GGAAKKTVVGSPDDPFYHKLSELLQGS	50.5	29.5	8.6	3.4	0.3
B6Hα-3-F6-IR	CGVGEQMEVTDDGDDPFYHKLSELLWSC	48.9	19.7	5.7	3.5	0.3
B6Hα-3-F3-IR	SGEQTATIEGPSNDPFYHKLSELIWGS	18.1	15.6	4.3	3.6	0.3
B6Hα-4-G8-IR	GGTKAVAKVGTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6	0.3
B6Hα-2-D1-IR	GCEVIVEEGDSADPFYHKLSELCQGS	11.7	5.4	1.3	4.2	0.2
B6Hα-3-E7-IR	GCAVVEEAERSRGDPFYHKLSELIQGC	47.0	5.6	1.3	4.3	0.2
B6Hα-2-D6-IR	GRTMAVMAAGGPDDPFYHKLSELVQGG	33.5	4.4	1.0	4.4	0.2
B6Hα-3-F10-IR	GCVVEWQKWHGASDPFYHKLSELGGCS	47.2	8.8	1.9	4.6	0.2
B6Hα-3-E8-IR	RGKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8	0.2
B6Hα-2-C10-IR	SGAKVIVVTGDSGDPFYHKLSELLQGS	46.9	5.8	1.1	5.3	0.2
B6Hα-2-C7-IR	RGIVAMVEATEVGSDHDPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1

FIG. 2H-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	--	--	--	--	--
B6L-4-G6-IGFR	AETPAQVGGDRLWSVWPGEHWNTVDPFYHKLS?LLRESGA	7.3	22.1	--	--	--
B6L-4-G10-IGFR	AE?PAQVGWNRLWSVWPGE?WNTVDPFYLKLSELL?ESGA	5.5	21.8	--	--	--
B6L-4-G3-IGFR	AET?AQVGWNG?WSVWPGEHWNTVDPFYYKLSELLRESGA	5.8	18.1	--	--	--
B6L-3-F10-IGFR	AETPAQVQWNRLWSVWPKDHWNTVDPFYHKLSELLRESGA	6.7	17.4	--	--	--
B6L-4-D2-IGFR	AETRAQVGSNRVWSVWPGEHWNTVDPFYHKLSELLRESGA	6.5	15.9	--	--	--
B6L-3-H10-IGFR	AETPAQVGWNRLWSVQPGSDWNTVDPFYHKLSELLRESGA	5.9	15.2	--	--	--
B6L-4-B12-IGFR	DETSAQVGWNRLWSDWPGEQWNTLDPFYHKLSELLRESGA	6.3	14.8	--	--	--
B6L-3-A9-IGFR	GETPAQVGWNRLWSVWPAEHWSTVDPFYHKLSELLRESGA	5.2	14.8	--	--	--
B6L-4-C4-IGFR	AETAAQVGWNRLQSVWPGEHWNSVDPFYHKLSELLRESGA	5.4	11.9	--	--	--
B6L-4-E3-IGFR	AEAPDQVGQNRLWSVWPGEHWNTVDPFYDKLSELLRESGA	4.4	11.1	--	--	--
B6L-4-A12-IGFR	PETPAQVGGNRLQSGWPGEHWNTVDPFYHKLSELLRESGA	3.0	10.4	--	--	--
B6L-4-D5-IGFR	AQTPAQVGWNYRLSSVWPGEHWNTVDPFYHKLSELLRESGA	4.7	10.3	--	--	--
B6L-3-A10-IGFR	AETPAQVGWNRLWSVQPGEHWNTVDPFYHKLSELLRQSDA	10.9	9.7	--	--	--
B6L-3-B9-IGFR	AETPAQVGWNRLWSVWPGEH*NTVDPFYHKLSELLRESGA	2.9	9.1	--	--	--
B6L-3-H9-IGFR	AETPAQVGWNSLQSVWPGEHWNT?DPFYHKLSELLRESGA	4.5	8.8	--	--	--
B6L-4-A5-IGFR	AESPAQVGSNRLQSVWSGEHWNTVDPFYHKLSELLRESGA	2.4	8.0	--	--	--
B6L-3-H11-IGFR	AETPAQVGQYRLSSVWPGEHGNTVDPFYHKLSELLRESGA	4.2	7.5	--	--	--
B6L-3-G10-IGFR	AETPAQVGWNRLWSVWPGEHWNTIDPFY*KLSELLRESGA	2.5	7.4	--	--	--
B6L-4-D4-IGFR	DETPAHVGWNRPQSAWPGERWNTVDPFYHKLSELLRESGA	2.4	6.8	--	--	--
B6L-4-F11-IGFR	AGTPAQVGWNRLRSVQPDEHWNTVDPFYHKLSELLRESGA	2.1	6.4	--	--	--
B6L-4-F12-IGFR	AETPAQVGWQRLWSVWPGEHWNP?DPFYRKLSELLRESGA	2.7	5.9	--	--	--
B6L-4-E12-IGFR	AETPAQVGWNRLQSVWPGEHWNTVDPFYHKLSELLRESGA	1.9	3.9	--	--	--
B6L-4-E10-IGFR	AETPAQVGWNRLWSVQPGEHWNTVDPFYHKLSELLRESGA	1.8	3.6	--	--	--
B6L-3-G9-IGFR	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1.2	2.5	--	--	--

FIG. 2G

Clone Parental/Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	--	--	--	--	--
B6L-4-C8-IR	ANLRLR?VGNRL*SVWPGEQWNTVDP?YQKLYELVRESGA	40.5	6.1	40.8	0.2	6.7
B6L-4-B7-IR	AETPAQVGWNRLWSVWPGEHWNIVDPFYHKLSELLRESGD	19.6	4.0	23.5	0.2	5.9
B6L-3-H1-IR	AETPAHVC*TVGGLFGRVNTWNTVDPFYAKLSELLRESGA	20.6	2.8	2.7	0.9	1.1
B6L-4-E12-IR	GQNSGSAWDGISLWSVWPGDVWNPVDPFYHKLSELLRESGA	15.5	4.1	3.0	1.1	0.9
B6L-4-D8-IR	AEAPAQVGWNLLQPGEHWITVDPFYNKLSELLRESGA	36.0	9.4	8.5	1.1	0.9
B6L-4-F7-IR	AETPAQVGWNGLWSVWPGEH*NTVDPFYHKLSELLRESDG	37.8	24.6	20.6	1.2	0.9
B6L-4-B11-IR	AETPAQVGQNGLWSVWPGEHWNTVDPFYQKLFELLRESGA	5.5	2.0	1.6	1.3	0.8
B6L-4-B12-IR	AETPAQVGQNRLWSVWPGELWNTVDPFYHKLSELLRESGA	6.8	2.0	1.6	1.3	0.8
B6L-4-B8-IR	T*QGETPAQVSLWPGEHWNTVDPFYHRLSELLRESGA	36.4	18.7	14.2	1.3	0.8
B6L-4-E8-IR	QGETPAQIGWNPLWSGWPGEHWNTLDPFYRKLSELLRESGA	35.6	11.4	8.6	1.3	0.8
B6L-3-G6-IR	VDTPAQVGWNRLWSVGPGEHWYTDDPFYH*LSELLRESGA	7.6	2.5	1.8	1.4	0.7
B6L-3-G5-IR	AETSAQVGWQRLWSVWPGDHWSTLDPFYHKLSELLRESGA	11.5	2.0	1.4	1.4	0.7
B6L-4-E10-IR	*NSPRVGWNGLWSVWPGEHWNTWDPFYNKLSELLRESGV	14.8	3.2	2.2	1.5	0.7
B6L-4-F10-IR	AETPAQIGWNRLQSVWPGEYWNTVDPFYLKLSELLRESSP	26.2	11.5	7.2	1.6	0.6
B6L-3-F3-IR	ADTPAQVSGNRLQSVWRGDPWNTVDPFYHKLSELLRESGA	36.0	17.1	10.1	1.7	0.6
B6L-4-A7-IR	AGTPAQVG*NRLWSVWPGEHWNTVDPFYNKLSELLRESGA	11.6	3.4	1.9	1.8	0.6
B6L-4-G8-IR	D*QAWSVWPGQHWNTIDPFYHKLSELLRESGA	30.4	11.2	5.9	2.0	0.5
B6L-4-F8-IR	AETLARVGWNRMQSVWPGEHWNTVDPFYHKLSELLRESGA	35.6	12.8	7.2	2.0	0.5
B6L-4-G7-IR	AATRPQVGWNRVWSVQPGEHWNTVDPFYHKLSELLRESGS	33.5	12.9	6.4	2.3	0.4
B6L-3-F4-IR	LTTPAQVGWNRLQSVWPGEHWNSVDPFYHKLSELLGESGA	16.9	6.3	2.7	2.4	0.4
B6L-3-H4-IR	ADNPAQVGWNRLWSVWPVEH*NTVDPFYHKLSELLRESGA	20.6	4.9	2.0	2.5	0.4
B6L-3-A6-IR	AETPAQVGWNRLQSDWPGGHWNTLDPFYHKLSELLRESGA	22.4	6.3	3.2	3.2	0.3
B6L-4-D7-IR	AETSVQVGWIRLQSVWPGEHWNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6L-3-E2-IR	G*NSAHVGWNRLWSFWPGEHWNTVDPFYEKLSELLRVSGG	29.2	16.7	3.8	6.3	0.2

FIG. 2F

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXCXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
20C-3-G3-IGFR	TFYSCLASLLTGTPQPNRGPWERCR	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	FFYDCLAALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
20C-3-F6-IGFR	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20C-3-A1-IGFR	SSYGCDGFYLMLFSLGLVASQELEC	26.5	20.8	1.5	13.7	0.1
20C-3-A4-IGFR	QFYGCLLDLSLGVPSFGWRRRCITA	17.7	8.8	1.2	7.6	0.1
20C-3-E4-IGFR	FFYRCLSRLLGGQLGSRLGLSCIGD	37.7	7.7	1.3	6.0	0.2
20C-4-D11-IGFR	DLFYCMMMQLATAGVGGSLGGPVCG	33.3	17.2	6.1	2.8	0.4
20C-4-F7-IGFR	CDFYCALSRLSGQPRDRMPNYPGTS	31.2	28.9	17.0	1.7	0.6
20C-3-B2-IGFR	GSACDGFYACLHALVQGPGEW	37.7	35.2	30.9	1.1	0.9

FIG. 2E

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
R20α-4-20C11-IR	DRAFYNGLRDLVGAVYGAWD	43.7	30.8	3.0	10.3	0.1
R20α-3-20E2-IR	FYDAIDQLVRGSARAGGTRD	46.3	39.9	3.1	12.9	0.1
R20α-4-20A12-IR	RLFYCGIQALGANLGYSGCV	48.6	39.9	2.4	16.6	0.1
R20β-4-C6-IR	FYSALWGLCGVTGCG	18.5	28.9	4.3	6.7	0.1
R20β-4-A6-IR	RGQSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1

FIG. 2C

Clone D sign	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
R20-4-F11-IGFR	GFYELLGALVGERVRGTGNS	39.0	19.2	--	--	--
R20-4-C7-IGFR	ERTDPFYKALLSLLGGDGSG	33.4	17.5	--	--	--
R20-3-F2-IGFR	DVQGNGSSGFYDGIFGLAWG	31.8	14.3	--	--	--
R20-4-A11-IGFR	PFYVWIRDLLGPELPHTRGD	37.8	13.5	--	--	--
R20-4-B12-IGFR	VLVVGGPLDPFYEGLHRLIS	37.2	10.0	--	--	--
R20-4-B10-IGFR	GFYRLLNELVREGGALKVGA	37.0	9.5	--	--	--
R20-4-E9-IGFR	GQRGFYELLSELLGHEGGVF	34.2	9.4	--	--	--
R20-3-H4-IGFR	DWVSGPFYRGIELLSGFQIE	30.3	7.8	--	--	--
R20-3-G2-IGFR	GGSLFYEGLLRLVLGDSVVG	20.8	6.9	--	--	--
R20-4-B8-IGFR	LNHFYAMLSDLSGVRNIFPG	32.8	6.5	--	--	--
R20-4-E7-IGFR	LSGFYEGLFRLARRDGSGWG	35.4	6.4	--	--	--
R20-4-G9-IGFR	FYDVLSALVGVELGEQGDAS	25.0	6.4	--	--	--
R20-4-D9-IGFR	GAGSFGREGGFYEALMQLAG	23.4	6.3	--	--	--
R20-4-D11-IGFR	DDEFYSQILKLVDGSRGGRSGTQN	31.3	4.0	--	--	--
R20-4-G10-IGFR	PFYMLLSRLVGGVEQEGGL	13.6	3.3	--	--	--
R20-4-C8-IGFR	FYDAIDQLVRGSARAGGTRD	16.8	3.2	--	--	--

FIG. 2D

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-40H4-IR	RYFPFGGFYGNLDVLRWLRPYVASPRWGHWRPGGSLGKQPT	31.9	1.4	2.1	0.7	1.5

FIG. 2A

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-B6-IGFR	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	--	--	--	--	--
R40-X-E5-IGFR	RHLTNAELGVQSPEVLSRLFPDGDIFYRALSHLVRGMGPP	--	--	--	--	--
R40-X-B5-IGFR	RGGMDRQWLDVGARHRLERRSVQDNTDDFYGGLRILVDGF	--	--	--	--	--
R40-4-9-IGFR	GPPDSFDVTEKGDMAILNVRFDPHSLDFNDQTFYFLLDSL	--	--	--	--	--
R40-3-G6-IGFR	GGTYFRGQVAQSNESLLRVNFLQLLEALAASPPRT	--	--	--	--	--
R40-4-12-IGFR	APFDARLSAPRFQWSPRTWXQSLSYGEWSCGSFYDCLSSI	--	--	--	--	--
R40-3-A5-IGFR	MGSSQFQDTRPSSGQAYSHSLDSDGWGTANWIFLRALEGL	--	--	--	--	--
R40-X-C6-IGFR	SGAAHEGNQGRERSTHLAANINDHLPGDAGIWLGYSWLS	--	--	--	--	--

FIG. 2B

Clone Design	Sequence X_n-FyxWF-X_m	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2Bα-4-H5-IR	RSNDDAFYRWFSNILQVDGGG	38.7	35.1	32.3	1.1	0.9
20E2Bα-4-G3-IR	DSDGAQFYIWFEDQLRSAGWD	35.5	36.1	32.7	1.1	0.9
20E2Bα-4-H4-IR	PGLHRAFYQWFAEAVRSANKE	38.8	37.9	35.0	1.1	0.9
20E2Bα-3-C1-IR	SLGQGGFYDWFASQVGGADI	43.7	42.1	39.0	1.1	0.9
20E2Bα-4-E6-IR	CGQTQSFYQWFCEVMRVESGD	38.0	34.3	29.7	1.2	0.9
H5-3-D5-IR	IVVPGDTQGVNFYIDWFVKQLQ	43.8	21.8	18.2	1.2	0.8
JBA5-3-D9-IR	RDVSMGSASTNFYIDWFVQQLG	38.3	29.8	25.3	1.2	0.8
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTVHSA	22.4	6.2	1.9	3.3	0.3
20E2Bβ-4-H10-IR	SNGISGFYEWFAAQVQTSDFQ	23.5	32.2	9.7	3.3	0.3
rB6-4-G8-IR	RRDRGGLDVFFYQWFMD	--	--	--	--	--

FIG. 10-3

Clone Design	Sequence X_n-FyxWF-X_m	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2Bα-3-D7-IR	TMGTQGFYRWFQNVVKEHLSG	35.4	26.9	31.3	0.9	1.2
20E2Bα-3-A12-IR	ITHNRGFYSWFLDVVQGGAGA	31.7	22.0	23.3	0.9	1.1
20E2Bα-3-D10-IR	VRRDAGFYQWFADILTQLDFE	32.7	27.3	29.1	0.9	1.1
20E2Bα-4-G7-IR	MQLQDEFYNWFRGIMLNDGQD	34.2	29.0	30.7	0.9	1.1
20E2Bα-4-F5-IR	GIRSSGFYQWFDRVLAGVGDG	33.8	32.1	34.0	0.9	1.1
20E2Bα-3-C9-IR	ANLNSQFYSWFASVTGEASPS	39.4	33.2	35.5	0.9	1.1
20E2Bα-3-A4-IR	QSPRASFYGWFDDVLRAAGVV	38.2	31.6	35.9	0.9	1.1
20E2Bα-4-E12-IR	MQRNQGFYSWFDDLVSSTVGV	36.0	30.8	29.7	1.0	1.0
20E2Bα-4-E11-IR	ASGFDPFYAWFLEQLRVANGS	35.1	31.2	30.7	1.0	1.0
20E2Bα-4-E8-IR	SGTPYGFYRWFQSALASATSG	36.1	30.5	30.7	1.0	1.0
20E2Bα-4-H10-IR	QGVEGGFYEWFDRAMGDVRPW	38.9	30.6	30.7	1.0	1.0
20E2Bα-4-F6-IR	DNMSGGFYRWFAQVVADSGGD	34.9	33.2	32.0	1.0	1.0
20E2Bα-4-G4-IR	RGTDDTFYGWFDQLLQGWCDD	34.1	33.7	32.2	1.0	1.0
20E2Bα-4-F8-IR	TVDHTQFYDWFSRVLGESGSA	37.7	32.0	32.7	1.0	1.0
20E2Bα-4-G5-IR	GRQDREFYYWFELQAGGMDGD	34.9	33.9	33.4	1.0	1.0
20E2Bα-3-B10-IR	RLLLGGFYEWFDQVLKETKEV	38.2	34.9	33.6	1.0	1.0
20E2Bα-3-C7-IR	GVLSTGFYEWFALQLHGLAAG	37.6	34.2	34.8	1.0	1.0
20E2Bα-3-C5-IR	PAVGQSFYGWFEAVLRGSKAG	40.4	36.0	35.6	1.0	1.0
20E2Bα-3-B9-IR	SNGISGFYEWFAAQVQTSDFQ	39.6	35.8	37.1	1.0	1.0
A6L-4-F11-IR	LLGLSQAAYANFYDWFVSQLA	33.1	4.6	4.6	1.0	1.0
20E2Bα-3-C2-IR	VPNSWMFYNWFAEQIEGSEGE	44.1	40.0	38.1	1.0	1.0
20E2Bα-3-B2-IR	ARRADGFYDWFREQVSGSAVQ	43.1	40.1	39.0	1.0	1.0
20E2Bα-4-G2-IR	GVVEGTFYEWFDRLLGGVQGD	34.1	33.6	29.8	1.1	0.9
20E2Bα-4-H6-IR	SHLTDPFYQWFVDQLRAGVRG	39.4	36.0	31.9	1.1	0.9

FIG. 10-2

Cl n Design	Sequence X_n-FyxWF-X_m	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2A-3-B11-IR	GRFYGWFQDAIDQLMPWGFDP	24.6	1.4	23.6	0.1	16.8
20E2Bβ-3-E3-IR	IQGWEPFYGWFDDVVAQMFEE	23.0	0.9	15.3	0.1	16.3
rB6-3-F6-IR	RYGRWGLAQQFYDWFDR	40.9	1.0	13.3	0.1	13.3
rB6-4-F9-IR	RGRLGSLSTQFYNWFAE	34.1	1.0	12.6	0.1	12.6
20E2Bα-3-A8-IR	ASAYTPFYQWFADVVSEYMQQ	35.4	7.4	34.4	0.2	4.6
A6L-4-F6-IR	PYRMEGTEKWNFYDWFVAQLQ	28.9	4.1	18.1	0.2	4.4
20E2Bα-4-H9-IR	SAVHFQFYKWFDNLLPVPLSA	37.8	9.4	26.7	0.4	2.9
20E2Bα-3-B1-IR	VPVNKSFYRWFQLVLGGSDDW	41.8	12.9	36.8	0.4	2.9
20E2Bβ-4-F9-IR	QSPRASFYGWFDDVLRAAGVV	25.9	4.2	10.1	0.4	2.4
20E2Bβ-3-E9-IR	TGFYEWFYEQLHSRMLPNPLD	27.0	7.7	17.2	0.5	2.2
20E2Bβ-3-E10-IR	RRGVGGFYGWFSQQLQGMGVA	22.2	2.6	5.5	0.5	2.1
20E2Bα-3-C12-IR	SSQDRRFYRWFEQAIVGGRDG	39.0	6.7	12.0	0.6	1.8
20E2Bβ-3-C12-IR	TRGQLGFYNWFQQALSTSGMG	20.2	2.2	3.8	0.6	1.8
20E2Bβ-3-E7-IR	CADLNAFYQWFCGVLDRGSDH	9.2	1.2	1.9	0.6	1.6
20E2Bβ-3-E11-IR	TLIQDQFYWWFSDLLSAEPGD	20.7	1.3	2.1	0.6	1.6
20E2Bα-3-B11-IR	IDQLDAFYRWFDGVMLGMGDP	36.0	20.7	32.8	0.6	1.6
NNKH-4-G2-IR	RGGGTFYEWFESALRKHGAG	10.8	6.3	8.9	0.7	1.4
20E2Bα-3-A7-IR	RGLDQDFYRWFQNLVGVEYDR	19.0	4.2	5.5	0.8	1.3
20E2Bα-4-G12-IR	MQGHRGFYGWFARVLEQDRGW	37.0	22.3	29.5	0.8	1.3
20E2Bα-3-C11-IR	ERLHLRFYEWFDTVIGQDGSD	37.3	26.8	34.8	0.8	1.3
20E2Bα-3-C10-IR	MHVQSDFYHWFQSLLGQGGPD	37.7	24.8	30.5	0.8	1.2

FIG. 10-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
H2CB-4-H1-IGFR	HGVIRADHTGFYGWFSKQLSD	18.3	15.3	16.5	0.9	1.1
H2CB-4-F9-IGFR	LINA.VFRRGFYAWFEEQVSK	22.9	14.4	15.3	0.9	1.1
H2CB-4-E10-IGFR	LQRYIGFHDPFYDWFSRALSG	26.1	20.1	24.5	0.8	1.2
H2CB-4-F8-IGFR	MRTAELFHVGFYDWFDAQLMD	21.5	14.8	19.0	0.8	1.3
H2CB-3-A8-IGFR	WAPPDALHGQFYRWFQRQLDQ	20.7	14.7	18.2	0.8	1.2
H2CB-4-F1-IGFR	AVHAATFHDDFYRWFEQVVGS	22.2	14.6	18.8	0.8	1.3
H2CB-3-C6-IGFR	FDAVHGFDGGFYGWFKRELQR	15.7	7.8	10.2	0.8	1.3
H2CB-4-E11-IGFR	QAGGMEFHGAFYNWFLQQLSG	26.1	17.6	24.1	0.7	1.4
H2CB-3-D6-IGFR	GRSVSRMNAEFYQWFGHQLAA	21.6	13.0	18.8	0.7	1.5
H2CB-4-F3-IGFR	AAVNSLFHDEFYLWFQDQLDG	17.3	11.1	16.4	0.7	1.5
H2CB-3-A4-IGFR	QLGMDWFHADFYEWFLAQLPS	27.4	11.0	14.8	0.7	1.3
H2CB-3-B1-IGFR	RLAGSGIHEGFYGWFVDQLLA	20.0	11.0	15.2	0.7	1.4
H2CB-3-C5-IGFR	GREIGGVHDGFYDWFRQQSEQ	19.9	10.5	15.6	0.7	1.5
H2CB-4-F6-IGFR	VRSEQRFDSSFYQWFNDLLMS	18.6	10.1	14.6	0.7	1.4
H2CB-3-B8-IGFR	QSPYGFFHDGFYRWFLQQTGM	20.7	6.9	9.5	0.7	1.4
H2CB-3-C7-IGFR	FQCGAAFHVDFYRWFTCQEQF	16.2	1.8	2.5	0.7	1.4
H2CB-4-H7-IGFR	GAFGSEFHEQFYRWFEDALSF	21.8	14.1	22.7	0.6	1.6
H2CB-4-F5-IGFR	EHTSYQIHRQFYEWFDRALGR	12.9	4.0	7.2	0.6	1.8
H2CB-4-G1-IGFR	SGTAADLHSRFYGWFALQARE	20.4	10.3	19.7	0.5	1.9
H2CB-3-D11-IGFR	EGFGVLFHGQFYRWFQLQLDG	24.1	8.8	18.6	0.5	2.1
H2CB-3-D7-IGFR	QQSAGHPHSSFYLWFSELLGA	22.1	6.5	13.6	0.5	2.1
H2CB-3-C10-IGFR	YLQRAGFHRSFYGWFDQALRD	21.7	5.1	10.4	0.5	2.0
H2CB-4-E3-IGFR	MWLWATLHSDFYSWFEQVVSG	20.3	4.6	8.9	0.5	1.9
H2CB-3-C1-IGFR	GANALGFKDRFYEWFAAQLWD	22.3	6.7	15.7	0.4	2.3
H2CB-4-G2-IGFR	GSGLYVFHWGFYDWFEQQMGG	19.9	3.3	10.7	0.3	3.3
H2CB-3-A11-IGFR	LDKGWGFDLQFYRWFEAATRA	23.9	2.5	7.7	0.3	3.1
H2CB-4-G5-IGFR	QRSAVEFHADFYDWFLRLLTP	19.3	2.5	7.9	0.3	3.1
H2CB-4-F12-IGFR	DQRMGSFHGEFYRWFEETLLS	16.7	1.7	5.4	0.3	3.1

FIG. 1N-3

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
H2CB-4-H10-IGFR	GALSSLFDAAFYDWFNRQLEG	21.9	22.4	23.3	1.0	1.0
H2CB-4-H5-IGFR	KVDLRGFHDGFYGWFARQLAG	22.3	22.3	21.6	1.0	1.0
H2CB-4-G7-IGFR	CSGLQRCHDSFYSWFESVVRE	23.1	21.6	20.6	1.0	1.0
H2CB-4-F4-IGFR	DSLGISFHEGFYDWFRRQLDM	21.3	20.9	21.3	1.0	1.0
H2CB-3-D8-IGFR	SGVFNGTFYDWFRIQLGE	20.0	20.5	21.6	1.0	1.0
H2CB-4-E4-IGFR	GYREMRSDLGFYQWFRDQLGL	21.6	20.5	21.2	1.0	1.0
H2CB-4-E5-IGFR	SVFMQHDHVGFYAWFRSLMEE	22.0	19.9	20.9	1.0	1.0
H2CB-4-E8-IGFR	FRHITEVDRSFYGWFVEQLRG	21.1	19.7	20.7	1.0	1.1
H2CB-3-D12-IGFR	WAGGSDVDGSFYDWFQRLLAS	26.6	17.3	16.8	1.0	1.0
H2CB-4-G9-IGFR	GLQNVSFHSGFYEWFARQVSQ	21.6	14.5	15.2	1.0	1.1
H2CB-3-C8-IGFR	SRVSDPYHVGFYQWFEEVVRG	20.8	13.4	13.9	1.0	1.0
H2CB-3-A12-IGFR	MGGATFFHTGFYDWFAAQLQH	28.6	27.5	29.2	0.9	1.1
H2CB-3-B12-IGFR	RPASRPFHSGFYQWFADQLSH	27.8	25.2	27.1	0.9	1.1
H2CB-3-A9-IGFR	GLAPGNFHEDFYRWFQEQTLG	27.7	24.3	25.7	0.9	1.1
H2CB-3-A3-IGFR	TAAISDFNSLFYGWFEQLLSS	26.9	24.1	26.5	0.9	1.1
H2CB-3-B4-IGFR	LDEDLPQHAGFYGWFAEALGV	25.8	23.8	25.3	0.9	1.1
H2CB-4-E7-IGFR	ASHKSAFDDNFYRWFSMQLRD	24.6	21.6	24.0	0.9	1.1
H2CB-4-G6-IGFR	HTGAGDLHGAFYNWFLEQLGG	22.4	21.1	23.0	0.9	1.1
H2CB-4-E9-IGFR	RRGRDGFHGGFYDWFAAQLSD	24.3	20.7	22.0	0.9	1.1
H2CB-4-H2-IGFR	GNFREAFHADFYSWFERQLQS	21.6	20.2	21.9	0.9	1.1
H2CB-3-A10-IGFR	RDTLPAFHQHFYQWFEKQVSA	24.3	19.9	21.5	0.9	1.1
H2CB-3-C4-IGFR	ERETAAFGQAFYQWFRDQIAG	23.1	19.2	22.0	0.9	1.1
H2CB-3-B5-IGFR	WGEGGGFYDWFYDQLGWEPSH	24.2	18.8	20.7	0.9	1.1
H2CB-4-G4-IGFR	SLVAADLHEGFYGWFRSQLGG	21.7	18.7	21.2	0.9	1.1
H2CB-3-D9-IGFR	TSEVGDFHAEFYSWFEIQLGR	24.4	18.6	20.0	0.9	1.1
H2CB-3-C3-IGFR	TGADGLLHARFYAWFEEQLRE	20.3	18.4	21.1	0.9	1.1
H2CB-3-D3-IGFR	RRSDSSLHRSFYDWFSVQLLN	22.5	18.3	21.3	0.9	1.2
H2CB-4-F2-IGFR	SESKYLLHSGFYGWFEAQLRG	18.0	16.8	18.3	0.9	1.1

FIG. 1N-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	**XXXXXXFHXXFYXWFXXXXXX**	--	--	--	--	--
Parental	**VTFTSAVFHENFYDWFVRQVS**	**29.8**	**17.5**	**16.3**	**1.1**	**0.9**
H2CB-3-D2-IGFR	TASQECFDDGFYGWFRAWRCT	22.9	18.6	11.8	1.6	0.6
H2CB-3-C12-IGFR	SLDWRWSEEPFYRWFQRALAG	17.3	19.6	13.0	1.5	0.7
H2CB-3-B11-IGFR	CMSLSDCHRKFYGWFKSQGGE	24.6	17.1	11.9	1.4	0.7
H2CB-4-E2-IGFR	LALCRRSPGSFYGWFQAAVGC	22.4	21.0	16.5	1.3	0.8
H2CB-3-A5-IGFR	PRSATMSDGGFYWWFASQLGL	28.8	26.1	22.6	1.2	0.9
H2CB-4-G12-IGFR	LRRSSVFHDPFYE*ISRLVGG	23.7	23.8	19.4	1.2	0.8
H2CB-3-B2-IGFR	ARLQQQFHGGFYEWFRAQVSP	23.0	19.9	16.4	1.2	0.8
H2CB-3-D1-IGFR	AQLDNLCHEPFYSWFCAVTRE	21.5	19.5	15.7	1.2	0.8
H2CB-3-B6-IGFR	WTCDTAFHQDFYQWFCDKLGV	16.3	4.5	3.7	1.2	0.8
H2CB-4-F7-IGFR	GKEGFGLDRDFYWWFREQLGP	22.0	19.0	18.0	1.1	0.9
H2CB-4-G8-IGFR	GRAPSSFDCDFYCWFRNQVQS	20.2	18.6	16.5	1.1	0.9
H2CB-3-D4-IGFR	DVEAETQHRLFYAWFLSQLGS	21.9	18.3	16.9	1.1	0.9
H2CB-3-D5-IGFR	ISVTAVFHDGFYGWFNEQVSK	21.4	17.9	16.4	1.1	0.9
H2CB-4-E6-IGFR	NSEHGRLDVDFYGWFARVIQQ	19.6	15.8	14.8	1.1	0.9
H2CB-3-C2-IGFR	GPLGDGCQDGFYGWFMCQVST	18.8	12.2	10.8	1.1	0.9
H2CB-3-A6-IGFR	KRSAYNFHDPFYDWFRMQLSG	26.8	29.0	28.1	1.0	1.0
H2CB-4-H12-IGFR	ASEPGGYLDPFYGWFREQLRA	23.9	28.3	28.1	1.0	1.0
H2CB-3-B10-IGFR	NRGDGGVHSGFYNWFRLQLSG	27.1	27.5	27.3	1.0	1.0
H2CB-4-F11-IGFR	ASKGSSLHNDFYGWFAQQLAR	25.5	25.5	24.6	1.0	1.0
H2CB-4-G11-IGFR	ANVSMWIQVGFYDWFDAQLRQ	25.3	25.4	25.3	1.0	1.0
H2CB-4-E12-IGFR	RTSPGSLHDPFYDWFQQQLGG	27.8	24.9	24.7	1.0	1.0
H2CB-4-G10-IGFR	PGVMSSFHGGFYSWFREQLNG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B9-IGFR	CLANSEDHDSFYGWFCQALGG	25.6	23.3	23.7	1.0	1.0
H2CB-3-B7-IGFR	GGSMGGMHGSFYEWFALQLRS	24.0	23.2	23.5	1.0	1.0
H2CB-4-H4-IGFR	RPQGGSIHAGFYQWFRDAVAG	23.5	23.1	23.8	1.0	1.0

FIG. 1N-1

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CBβ-3-E8-IR	TGHRLGLDEQFYWWFRDALSG	15.9	1.9	11.8	0.2	6.1
H2CBβ-4-F8-IR	VLTSNTLHQRFYSWFAAARRE	13.4	0.8	2.6	0.3	3.4
H2CBβ-3-C4-IR	CVAQGGFQSSFYCWFAGLDID	21.1	1.3	4.0	0.3	3.1
H2CBβ-3-D5-IR	NGQSSRFHTAFYDWFAAQLSG	14.0	3.3	10.2	0.3	3.1
H2CBβ-3-E6-IR	SVPRGTVHDAFYQWFREVALG	5.7	0.7	2.1	0.3	3.1
H2CBβ-4-G12-IR	GARGSTFHDQFYEWFWVQLGD	6.8	1.8	5.4	0.3	3.1
H2CBβ-4-F4-IR	PPGMNGFHTSFYSWFVDQLGD	17.9	1.9	5.6	0.3	3.0
H2CBβ-4-F11-IR	AVGTLGYHSGFYRWFERQLGG	15.0	1.7	4.8	0.3	2.9
H2CBβ-3-E5-IR	ELQARGVHRNFYRWFEAQVSG	17.0	1.8	5.0	0.4	2.8
H2CBβ-4-F2-IR	HRVARAFHEQFYDWFEKAVSG	15.9	1.3	3.4	0.4	2.6
H2CBβ-4-G4-IR	GAMEPDYHRSFYQWFAAALGE	8.7	1.4	3.5	0.4	2.6
H2CBβ-3-C8-IR	CPDRQSVDDRFYNWFADALAS	4.9	1.4	3.2	0.4	2.3
H2CBβ-4-F10-IR	GGAQISFHERFYQWFLQEAAG	10.2	1.0	2.4	0.4	2.3
H2CBβ-4-H4-IR	HKRGIVQHGAFYAWFDSLLSG	20.8	4.2	9.5	0.4	2.3
H2CBβ-4-G6-IR	QASDNRSDGQFYLWFEKLLSS	14.5	5.6	8.5	0.7	1.5
H2CBβ-4-H1-IR	DRGRMGVDEGFYNWFARQMQE	17.0	10.1	13.2	0.8	1.3

FIG. 1M-5

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CBα-3-D6-IR	AGVMGGFHQEFYLWFERALSN	27.9	26.0	25.8	1.0	1.0
H2CBα-3-H3-IR	AGHVGQVYDGFYGWFREQLGA	27.0	26.9	26.2	1.0	1.0
H2CBα-3-F4-IR	FVQNIGFDYDFYGWFVREVEK	31.2	27.2	27.7	1.0	1.0
H2CBα-3-E9-IR	PVGIGGLHRAFYQWFQSQVDA	31.6	27.7	28.2	1.0	1.0
H2CBα-3-H10-IR	GSRQEADHQAFYDWFNLVLGV	26.9	27.9	28.8	1.0	1.0
H2CBα-3-G2-IR	AGGRKPFHDDFYGWFRDQLAE	29.1	28.1	28.8	1.0	1.0
H2CBα-3-B2-IR	DLASHGFHDAFYNWFSVQLNS	29.4	28.1	28.2	1.0	1.0
H2CBα-3-E8-IR	GSNGGGVHGQFYAWFVEALSG	31.5	28.4	29.1	1.0	1.0
H2CBα-3-E5-IR	RGRASTFHDGFYGWFSQQLRF	33.0	28.7	28.9	1.0	1.0
H2CBα-3-E6-IR	SPARRVSHHDFYGWFAKQLES	29.6	29.0	28.1	1.0	1.0
H2CBα-3-E7-IR	SSDVGAFHSAFYDWFKAQLSG	30.4	30.2	30.2	1.0	1.0
H2CBα-3-C8-IR	PTVHRAFDDLFYGWFAKQVED	31.9	31.2	31.5	1.0	1.0
H2CBα-3-A4-IR	SSNTVGLDERFYAWFVDQLGA	32.2	31.9	32.6	1.0	1.0
H2CBα-3-D1-IR	PGAAEGFHSAFYDWFAQAVSG	32.9	32.5	31.5	1.0	1.0
H2CBα-3-B9-IR	MRSEASFHVEFYSWFEEQLRS	33.2	33.8	33.3	1.0	1.0
H2CBα-3-D8-IR	VSRYGGQQDGFYHWFSDLLKG	26.3	20.2	19.1	1.1	0.9
H2CBα-3-F1-IR	RPSSGGLHYGFYHWFRVQEEM	28.8	28.0	26.4	1.1	0.9
H2CBα-3-A11-IR	SNIEEHFHMQFYRWFSDALGN	20.5	21.5	17.7	1.2	0.8
H2CBα-3-A3-IR	ANDCLGLHAGFYGWFACQLGG	30.4	29.6	21.8	1.4	0.7

FIG. 1M-4

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CBα-3-A9-IR	RVDAAALNAGFYEWFRGVIQG	30.5	21.7	24.1	0.9	1.1
H2CBα-3-C11-IR	GGAGRSFHDAFYEWFERQMAG	26.4	21.8	23.2	0.9	1.1
H2CBα-3-B4-IR	EGARQGFHARFYSWFAQQLAL	30.9	22.0	24.3	0.9	1.1
H2CBα-3-F11-IR	VLLPGVVHGGFYDWFSRQLSS	24.5	22.5	23.9	0.9	1.1
H2CBα-3-G10-IR	GALSDRYNNVFYDWFREQLLG	28.3	23.6	27.1	0.9	1.1
H2CBα-3-D7-IR	PDSFMSLHQRFYSWFQAQVGT	31.4	23.6	25.3	0.9	1.1
H2CBα-3-E2-IR	RVYKANFHNEFYGWFREQLLG	26.8	24.0	25.7	0.9	1.1
H2CBα-3-B5-IR	HSGMRDVHARFYSWFSEQLSG	28.7	25.0	26.4	0.9	1.1
H2CBα-3-C7-IR	ARLLERFQDPFYEWFETLMGD	30.0	25.2	28.7	0.9	1.1
H2CBα-3-G9-IR	RNSSGNFHDKFYNWFEAQLKG	27.8	25.2	26.7	0.9	1.1
H2CBα-3-A12-IR	GSMSPVFNDQFYGWFRDLVDE	28.0	26.4	28.7	0.9	1.1
H2CBα-3-C9-IR	SCTGRQFDGCFYAWFEDQLVG	32.1	28.7	31.9	0.9	1.1
H2CBα-3-B10-IR	GIAVQSLHDSFYRWFDNALGS	33.5	30.8	33.2	0.9	1.1
H2CBα-3-E1-IR	IGPPGSLHRGFYDWFAEQVEA	31.7	30.5	29.0	1.1	1.0
H2CBα-3-G12-IR	GAAGISFHRGFYDWFAAQVRD	29.1	31.4	29.8	1.1	1.0
H2CBα-3-F7-IR	GVDVTDFHKDFYSWFQRQLNG	23.2	20.7	20.3	1.0	1.0
H2CBα-3-G8-IR	WAGRAGIHGGFYEWFNRQLRG	22.8	20.9	20.4	1.0	1.0
H2CBα-3-C6-IR	LGQLAAFHLGFYEWFSEAVAA	26.7	21.2	22.0	1.0	1.0
H2CBα-3-H9-IR	VHSVSRLNVGFYQWFQDQLSG	23.4	22.5	22.0	1.0	1.0
H2CBα-3-H8-IR	LGLMAIFDRGFYGWFEQQLSG	23.5	23.4	23.2	1.0	1.0
H2CBα-3-F2-IR	VARGSSLHDDFYEWFASQLRT	25.5	24.3	25.2	1.0	1.0
H2CBα-3-D5-IR	LGYIGALNTQFYSWFADLVGS	26.7	24.5	25.6	1.0	1.0
H2CBα-3-D10-IR	EDSRLRLHEGFYGWFRKQLGD	26.8	24.9	24.9	1.0	1.0
H2CBα-3-F10-IR	GRDNMKFHSGFYDWFTQQLAG	25.7	25.6	26.1	1.0	1.0

FIG. 1M-3

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
H2CBα-3-D11-IR	GPRGQRLHDAFYSWFDALRVN	27.8	13.0	24.8	0.5	1.9
H2CBα-3-H12-IR	LGTLAVFHELFYGWFERQLGG	27.4	7.2	12.4	0.6	1.7
H2CBα-3-A10-IR	LGGYCGFNCQFYRWFDNLADR	27.1	13.2	22.3	0.6	1.7
H2CBα-3-A5-IR	FSGWADYQSGFYQWFAEELAN	28.3	16.1	28.1	0.6	1.7
H2CBα-3-C4-IR	WGPFSVFDESFYRWFAQASDD	30.7	17.2	29.2	0.6	1.7
H2CBα-3-B8-IR	PRNEGLVHGLFYDWFQRALSG	25.6	11.3	18.6	0.6	1.6
H2CBα-3-H11-IR	DEGGAPLDVMFYRWFEQAVRG	28.8	14.0	22.4	0.6	1.6
H2CBα-3-E10-IR	QSGNRGSHGAFYSWFRDVLAN	27.7	14.3	23.0	0.6	1.6
H2CBα-3-C2-IR	MRQRDGFNSSFYGWFAAALGE	28.4	17.0	26.7	0.6	1.6
H2CBα-3-F6-IR	SEERKKVHSQFYSWFDRQLLG	27.3	14.5	21.8	0.7	1.5
H2CBα-3-D4-IR	PSPNAPFHGGFYDWFDWVQGS	29.0	18.9	27.1	0.7	1.4
H2CBα-3-A7-IR	FHRPGSFNTNFYQWFDDQMNQ	29.1	19.4	26.9	0.7	1.4
H2CBα-3-H4-IR	SDDSSTLNGRFYTWFHMQLLD	27.2	20.1	27.9	0.7	1.4
H2CBα-3-B7-IR	QRGGGGFHEGFYSWFRSQSLL	28.6	18.0	23.6	0.8	1.3
H2CBα-3-F9-IR	SGSRPVFHEQFYEWFVDQLGL	26.1	19.1	24.3	0.8	1.3
H2CBα-3-H6-IR	GGSSQAFHGAFYEWFSAQLRG	24.8	21.6	27.3	0.8	1.3
H2CBα-3-F5-IR	AFVSERVNQRFYDWFRDQMRS	29.4	22.0	27.8	0.8	1.3
H2CBα-3-A2-IR	VRHPTRFHDEFYRWFTEQLTT	30.7	22.5	29.1	0.8	1.3
H2CBα-3-F3-IR	ARLLNIFDRGFYNWFQRQLDE	16.3	6.7	9.0	0.7	1.3
H2CBα-3-G6-IR	PSLSSNLHESFYRWFDQLVST	24.9	21.0	24.4	0.9	1.2
H2CBα-3-G7-IR	FAFGLGFHQGFYDWFAHQLEG	24.4	18.7	23.0	0.8	1.2
H2CBα-3-C5-IR	VSATVMLHREFYDWFGLQLLD	26.4	21.2	25.4	0.8	1.2
H2CBα-3-G1-IR	GGVSGVLHDRFYSWFERQLAG	26.9	21.5	26.3	0.8	1.2
H2CBα-3-E3-IR	GLGIASFHEGFYSWFTAQLGA	24.2	17.2	19.3	0.9	1.1

FIG. 1M-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CBα-3-B12-IR	QSDSGTVHDRFYGWFRDT*A	26.0	1.3	20.4	0.1	16.0
H2CBα-3-D2-IR	WTDVDGFHSGFYRWFQNQWER	20.6	1.7	12.1	0.1	7.0
H2CBα-3-D12-IR	VASGHVLHGQFYRWFVDQFAL	24.6	2.1	14.0	0.1	6.7
H2CBα-3-H5-IR	QARVGNVHQQFYEWFREVMQG	16.7	2.4	15.1	0.2	6.3
H2CBα-3-B6-IR	VGDFCVSHDCFYGWFLRESMQ	31.4	2.5	13.9	0.2	5.6
H2CBα-3-G11-IR	SGSRPVFHEQFYEWFVDQLG	22.7	1.4	6.4	0.2	4.7
H2CBα-3-A6-IR	QFSAGAFHGDFYGWFRALYNG	25.9	1.7	7.1	0.2	4.3
H2CBα-3-B1-IR	SRFDERLHHQFYEWFRVLNEP	33.4	6.0	25.5	0.2	4.3
H2CBα-3-F8-IR	DSVNSDLHRAFYGWFAEQWRA	23.0	4.8	19.8	0.2	4.1
H2CBα-3-E11-IR	GSVDREIHGPFYSWFSEQLWG	14.0	2.2	8.5	0.3	4.0
H2CBα-3-G4-IR	SAKTPVLHDGFYMWFEAQSES	24.9	2.2	6.9	0.3	3.2
H2CBα-3-D3-IR	LVVGRRFHQSFYDWFVAAAGG	23.6	2.6	8.0	0.3	3.1
H2CBα-3-C1-IR	IMWPCTFQDPFYCWFQTEQGR	27.0	5.6	16.4	0.3	2.9
H2CBα-3-C3-IR	VVGPLDIHERFYGWFHQQGGA	23.3	1.1	3.1	0.4	2.8
H2CBα-3-G3-IR	VVPKAGFHEAFYEWFRRQDRD	23.7	6.7	17.6	0.4	2.6
H2CBα-3-E4-IR	QSFVTSVHTRFYAWFASALEM	28.8	8.3	21.9	0.4	2.6
H2CBα-3-G5-IR	SRGLGLYHSGFYGWFERQFNQ	26.7	7.0	17.2	0.4	2.5
H2CBα-3-B11-IR	GADTGAVHRRFYLWFEQLSGG	28.0	8.6	19.4	0.4	2.3
H2CBα-3-A1-IR	PGNRPTFHAEFYRWFREAQGS	31.3	11.3	24.9	0.5	2.2
H2CBα-3-H1-IR	VAVAWGLHESFYAWFENQFSD	27.2	10.6	23.9	0.4	2.2
H2CBα-3-F12-IR	GFNTGTFHDQFYYWFWEAAGG	21.1	6.1	12.7	0.5	2.1
H2CBα-3-H7-IR	GDGLTAFHQGFYEWFDIQMYG	21.0	9.7	19.1	0.5	2.0
H2CBα-3-C12-IR	VGVNRQFHTRFYAWFDEQLGG	26.0	12.7	24.7	0.5	1.9

FIG. 1M-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
H2CA-3-E6-IGFR	WRGHGTFHEDFYDWFVRQVSGSGSST	15.7	8.7	3.1	2.8	0.4
H2CA-4-F4-IGFR	GGRVGVLHENFYDWFDRQVSLRGADG	11.5	7.4	3.0	2.5	0.4
H2CA-3-D10-IGFR	CNLTAGFHEQFYHWFAIQVCGDAENA	9.4	6.8	2.9	2.3	0.4
H2CA-3-E1-IGFR	ERGEDMFHENFYDWFVRQISGRQGGG	12.5	6.4	2.8	2.3	0.4
H2CA-2-B6-IGFR	TNQGVGFYDSFYGWFVRQIQYGVDSG	18.0	6.2	2.7	2.3	0.4
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2	0.5
H2CA-4-H2-IGFR	QTFGKSLHENFYDWFVRQVSREEGGD	9.8	9.9	4.8	2.1	0.5
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5
H2CA-2-B8-IGFR	SASTHQFHENFYDWFVRQVSGAQKIL	14.6	7.9	3.9	2.0	0.5

FIG. 1L-2

Cl ne	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
H2CA-4-F-IGFR5	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFLRQVAESSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFERQVSHQRGVR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDWFVRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYEWFVRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEWFVRQVSLSLKGK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
H2CA-4-F7-IGFR	SSIGGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	QSPVGSSHEDFYDWFFRQVAQSGAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDWFDRQVSQSGGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDWFVCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5	0.3
H2CA-3-D4-IGFR	RSASGSLPEQFYDWFVRQVSLSGTDK	17.6	13.8	4.1	3.4	0.3
H2CA-4-F2-IGFR	SRVTTVFHENFYDWFVRQLSDSAISG	9.3	12.8	4.2	3.0	0.3
H2CA-3-D11-IGFR	DERGGKFREDFYDWFVRQVSESRFGQ	12.2	6.9	2.3	3.0	0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3

FIG. 1L-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
H2CA-4-G7-IR	RAGGVGLHDNFYDWFVRQVSGGDSGP	35.9	34.7	23.7	1.5	0.7
H2CA-3-C6-IR	ADCYVQLHENFYDWFRRQVCNLQEGM	38.7	37.6	28.2	1.3	0.7
H2CA-3-B8-IR	RQGHAGFHDNFYDWFVRQVSGSTPQV	37.8	19.6	9.9	2.0	0.5

FIG. 1K-4

Clone Design	Sequence XXXXXXFHENFYDWFVRQVSXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CA-3-D12-IR	SEDVDSRHENFYDWFVRQVSGIGLQD	36.8	34.1	29.6	1.2	0.9
H2CA-3-B5-IR	PAPADAFDHNFYDWFARQLSATTTIQ	38.8	35.2	30.5	1.2	0.9
H2CA-4-E1-IR	MVQRISIHENFYDWFVRQISGSAVPP	29.8	12.5	11.3	1.1	0.9
H2CA-3-D3-IR	GNVRGQFHGQFYDWFARQVSGSEGDA	33.1	29.9	27.5	1.1	0.9
H2CA-4-E3-IR	PDAEKQFHETFYGWFVRQISEDSANS	33.3	32.3	30.2	1.1	0.9
H2CA-4-E12-IR	FGRGVHCDENFYDWFVCQVSGALLEG	36.0	32.4	29.4	1.1	0.9
H2CA-3-A6-IR	ETPLTELHEQFYDWFVRQVSGFPGGV	34.0	33.1	30.6	1.1	0.9
H2CA-4-E9-IR	QHRGPHFHEDFYDWFVRQVSSAVPSD	38.8	33.7	29.7	1.1	0.9
H2CA-4-F3-IR	RQDPGLFHDNFYDWFDRLVSAWDGQE	41.0	34.2	32.0	1.1	0.9
H2CA-4-H6-IR	QAAVGVCNKDFYAWFACQVREDFAKA	37.1	34.5	30.8	1.1	0.9
H2CA-4-H2-IR	RNWNLQFNENFYDWFDRQVSALRGGG	41.8	35.3	32.8	1.1	0.9
H2CA-3-D4-IR	RSEQYRFHENFYEWFDRQVSRMGLLG	38.7	35.5	32.3	1.1	0.9
H2CA-3-D1-IR	GAGGRDFDEDFYDWFVRQVSGQVTSG	34.5	35.5	31.3	1.1	0.9
H2CA-3-C1-IR	SPEGNLVHDQFYDWFVRQLSSTSAGT	39.9	36.1	32.9	1.1	0.9
H2CA-3-D8-IR	QGGLGDFDEDFYDWFARQVSRRDRAD	37.8	36.7	33.1	1.1	0.9
H2CA-4-H4-IR	LSQGVGFQENFYEWFERQVSGWDGRD	38.5	37.0	33.7	1.1	0.9
H2CA-4-F6-IR	VFERSRCHDNFYDWFFCQVSGQADGG	38.7	37.5	35.2	1.1	0.9
H2CA-4-E4-IR	LLASRAFHENFYDWFARQVSGTQPPG	38.6	38.0	34.7	1.1	0.9
H2CA-3-C11-IR	VPDAQIFHESFYDWFVRQASAGGPAD	40.3	38.3	36.1	1.1	0.9
H2CA-3-C4-IR	ANQMGRFHDNFYDWFDRQVSRYERGT	41.9	38.4	35.0	1.1	0.9
H2CA-4-E6-IR	PSRKDGLHQSFYDWFARQVQDMEGRA	39.3	38.8	35.8	1.1	0.9
H2CA-3-D7-IR	QAVTRRFHENFYDWFARQVSEEGGWS	42.5	39.2	35.5	1.1	0.9
H2CA-3-A7-IR	GYAVGQYQANFYDWFVRQVDGMSNGG	35.3	15.2	11.6	1.3	0.8
H2CA-4-G12-IR	GHQRDLLHESFYDWFVRQVSEAEGGG	37.6	19.4	15.1	1.3	0.8
H2CA-3-D6-IR	DRPSSFIHENFYEWFARQVSQSGSSG	39.4	36.2	27.6	1.3	0.8
H2CA-4-H12-IR	ERTAETLHEQFYDWFVRQVSAMDGES	40.0	38.4	29.3	1.3	0.8
H2CA-3-D11-IR	LTSQLLSHEDFYDWFVRQVSGVGGSG	38.1	32.9	27.2	1.2	0.8
H2CA-3-C12-IR	PDRSDRLDDNFYDWFVRQVSQVINED	38.5	38.4	31.7	1.2	0.8

FIG. 1K-3

Clon	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
H2CA-3-D9-IR	VSGRGAFHENFYDWFVRQVFRDEQDT	36.6	30.6	30.9	1.0	1.0
H2CA-3-C2-IR	ARPPPTVHENFYDWFVRQVSETWRQD	38.3	30.7	31.0	1.0	1.0
H2CA-4-G1-IR	QGGDRLFHERFYDWFDRLVSSDSTGE	34.1	30.7	30.4	1.0	1.0
H2CA-4-E2-IR	QHIAAGLHENFYDWFIRQVSGVNVPA	33.9	31.0	31.8	1.0	1.0
H2CA-4-H9-IR	QPNDGLLHENFYDWFVRQVSNAVDGG	38.9	31.1	31.4	1.0	1.0
H2CA-3-D2-IR	PVEFTVYHDNFYDWFARQVSDGLGQF	33.0	31.1	29.8	1.0	1.0
H2CA-3-B3-IR	FCVQASIHENFYDWFVRQVAENQVFS	35.3	31.4	30.0	1.0	1.0
H2CA-4-G11-IR	GRPRGSFHENFYDWFARQVSGDGAGT	37.9	31.9	31.0	1.0	1.0
H2CA-4-F2-IR	IVGASLCHESFYDWFACQVTNLQSQG	38.1	32.0	31.9	1.0	1.0
H2CA-3-C5-IR	IGLRQMFHENFYDWFAREVSKEAGDG	36.9	32.3	31.6	1.0	1.0
H2CA-3-B2-IR	LGGAIEGHGNFYDWFVRQVSLDVGGE	36.6	32.7	32.5	1.0	1.0
H2CA-3-B11-IR	LNALQQLHENFYDWFGRQVSATPPGG	35.5	32.8	33.3	1.0	1.0
H2CA-4-G2-IR	VGNCDTFPENFYDWFACQVSELGGMN	35.9	33.0	33.4	1.0	1.0
H2CA-3-A4-IR	FSQDGNFHENFYDWFDRQLSLVGAGT	33.3	33.0	32.9	1.0	1.0
H2CA-4-H3-IR	PAGNRALHESFYDWFVRQVSEFQLGA	39.5	33.7	33.7	1.0	1.0
H2CA-4-G5-IR	DRLRARFNENFYDWFDRQVSGQGSMP	35.3	34.0	35.6	1.0	1.0
H2CA-4-E8-IR	VLGVAQFHDKFYDWFARQVSQLESAG	35.7	34.7	34.9	1.0	1.0
H2CA-4-G6-IR	GVVGGAFHEQFYDWFDRQVSAAFKGD	36.2	35.0	33.5	1.0	1.0
H2CA-3-B7-IR	DESEMRLHEQFYDWFARLVSLEGGSA	37.6	36.5	35.3	1.0	1.0
H2CA-3-B4-IR	EGGGVAIHENFYDWFDRQVSLQGWSD	39.8	36.5	35.1	1.0	1.0
H2CA-3-C7-IR	SRIVSRFHENFYDWFVRQVSGDAPVQ	40.2	36.7	35.9	1.0	1.0
H2CA-4-E5-IR	IPAGAQLHENFYDWFARQVSGEDGGA	37.3	37.0	36.3	1.0	1.0
H2CA-4-E7-IR	GSSAAGFDEQFYDWFDRQVSEAFRDG	39.7	37.6	37.6	1.0	1.0
H2CA-3-B9-IR	RLALRTFHQDFYDWFVRQVAAEDTDP	39.4	37.7	37.6	1.0	1.0
H2CA-4-F5-IR	QGSFAVLHENFYDWFARQVSGVEGLA	38.8	38.0	37.8	1.0	1.0
H2CA-3-B10-IR	QGNMSALHENFYDWFVRQVSEADRVD	41.9	38.9	38.0	1.0	1.0
H2CA-3-A12-IR	VAYPALLHEQFYDWFVRQVSAVAGTT	37.8	7.3	6.3	1.2	0.9
H2CA-3-A8-IR	PDTINSQHKNFYDWFVRQVSGVGTSS	36.8	22.5	19.2	1.2	0.9

FIG. 1K-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	37.7	2.2	18.1	0.1	8.2
H2CA-4-E10-IR	QRLSLHEQFYDWFVGQVSPLGAGG	31.2	4.4	18.8	0.2	4.3
H2CA-4-G3-IR	GGGKVNFHEDFYGWFVQQFSGVGSDR	36.1	13.4	25.7	0.5	1.9
H2CA-3-A11-IR	LVGDAPFHEDFYDWFARQVFGCCQEQ	35.6	12.1	22.0	0.5	1.8
H2CA-4-F8-IR	TGAEVSFHENFYDWFDRQYSSWLDRD	36.0	21.1	33.5	0.6	1.6
H2CA-4-G4-IR	QPHSSRLHESFYDWFDRQVPWYALDR	37.1	23.3	34.3	0.7	1.5
H2CA-4-F4-IR	SRALAAVHEQFYDWFVRQVSGLDWGY	39.8	25.0	35.6	0.7	1.4
H2CA-4-H10-IR	QPKDGTLHENFYDWFVRQVSSSGWVG	33.5	5.1	6.6	0.8	1.3
H2CA-4-F1-IR	RGRLIQLHEDFYDWFLRQVSGMGGGS	36.1	19.6	25.1	0.8	1.3
H2CA-3-D5-IR	QRGAPKSDENFYDWFVRQVLRFGEND	39.3	24.3	31.9	0.8	1.3
H2CA-4-E11-IR	AARTSLFHEDFYEWFDRQVRQEGMWG	8.2	2.6	3.2	0.8	1.2
H2CA-3-B6-IR	GTSNHSLHENFYDWFVRQLSSVQSSG	35.9	9.9	12.1	0.8	1.2
H2CA-3-A9-IR	VSHVHLFHENFYDWFVRQLAAEGFSG	37.3	30.1	36.2	0.8	1.2
H2CA-4-H5-IR	GRQDSGLHEHFYDWFSRQVQGEVALG	38.6	35.4	37.3	1.0	1.1
H2CA-3-C9-IR	SNDERQFHETFYDWFVRQVSADGADR	29.3	5.1	5.6	0.9	1.1
H2CA-3-A10-IR	LSTEQRFHEKFYDWFVHQVSTSGGGT	37.2	16.9	19.1	0.9	1.1
H2CA-3-A3-IR	SLSREQFHENFYDWFARQVSELEGVV	29.2	28.6	32.2	0.9	1.1
H2CA-4-G8-IR	IPGRRSLHENFYDWFVRQVSPGGGSA	32.4	29.1	31.6	0.9	1.1
H2CA-4-G9-IR	TQKAQSLDEKFYDWFVRQVSGGGLTG	36.1	34.4	36.4	0.9	1.1
H2CA-4-G10-IR	VSQLSDFHENFYGWFARQIAGQAEWT	34.2	35.5	37.7	0.9	1.1
H2CA-4-H7-IR	NGTSQALHQNFYDWFAQQISGSEPGP	37.0	36.0	40.0	0.9	1.1
H2CA-4-F9-IR	VGQSVTFHGDFYDWFDRQLSGSQEFG	37.5	36.7	39.5	0.9	1.1
H2CA-4-F7-IR	TIDHHPLHEQFYDWFARQVSDLESLG	37.7	37.6	39.9	0.9	1.1
H2CA-3-D10-IR	PNVGYAFHENFYDWFIRQVSIEEKAG	18.7	3.6	3.5	1.0	1.0
H2CA-3-B1-IR	SRGSGVFHESFYNWFDRQVSEWIQFG	26.5	21.4	21.5	1.0	1.0
H2CA-3-A5-IR	QPVSGSVHERFYDWFVRQVSGSAGGG	32.9	22.9	22.4	1.0	1.0
H2CA-4-F10-IR	ASQLPPVYENFYEWFDRQVSLDAQRE	26.6	27.7	28.5	1.0	1.0

FIG. 1K-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	GFREGNFYDWFVAQVT	--	--	--	--	--
E4D-1B-B8-IGFR	GFREGDYYGWFEAQVT	23.8	10.7	--	--	--
E4D-1-G7-IGFR	GFREGDFYAWFMAQVT	14.3	10.5	--	--	--
E4D-1B-A11-IGFR	GFREGNFYEWFLAQVT	24.0	10.0	--	--	--
E4D-1-C3-IGFR	GFREGSFYDWFDAQVT	15.8	9.3	--	--	--
E4D-2-H1-IGFR	GFREGNFYDQFVAQVT	19.6	4.9	--	--	--
E4D-1-C2-IGFR	GFREGHFYEWFAAQVT	11.5	4.5	--	--	--
E4D-1B-A12-IGFR	GFREGNFYEWFVAQVT	18.4	3.5	--	--	--
E4D-1B-A1-IGFR	GFREGKFYDWFVAQVT	22.5	2.9	--	--	--
E4D-2-A3-IGFR	GFREGMFDVQLLAQVT	22.7	2.1	--	--	--

FIG. 1J-2

Clone Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	GFREGNFYDWFVAQVT	--	--	--	--	--
E4D-2-E7-IGFR	GFREGDFYDWFRAQVT	20.8	22.8	--	--	--
E4D-2-C11-IGFR	GFREGSFYDWFVAQVT	21.5	22.6	--	--	--
E4D-2-B1-IGFR	GFREGDFYGWFQAQVT	22.0	22.5	--	--	--
E4D-2-D10-IGFR	GFREGGFYDWFQAQVT	20.6	22.1	--	--	--
E4D-2-A9-IGFR	GFREGDFYDWFVAQVT	17.4	21.5	--	--	--
E4D-2-E5-IGFR	GFREGDFYDWFQAQVT	24.2	21.2	--	--	--
E4D-2-H9-IGFR	GFREGGFYDWFVAQVT	19.1	20.7	--	--	--
E4D-1B-C4-IGFR	GFREGDFYDWFAAQVT	24.3	20.5	--	--	--
E4D-2-E10-IGFR	GFREGNFYDWFQAQVT	21.0	20.5	--	--	--
E4D-2-F4-IGFR	GFREGNFYDWFLAQVT	25.0	20.2	--	--	--
E4D-2-C10-IGFR	GFREGHFYDWFQAQVT	22.8	20.1	--	--	--
E4D-3-D8-IGFR	GFREGQFYEWFEAQVT	21.1	19.8	--	--	--
E4D-3-F9-IGFR	GFREGSFYEWFQAQVT	22.6	19.7	--	--	--
E4D-1B-E5-IGFR	GFREGDFYDWFLAQVT	24.2	18.8	--	--	--
E4D-2-F3-IGFR	GFREGHFYDWFVAQVT	23.6	18.0	--	--	--
E4D-3-D5-IGFR	GFREGQFYEWFVAQVT	22.2	18.0	--	--	--
E4D-3-G10-IGFR	GFREGQFYDWFAAQVT	22.1	17.6	--	--	--
E4D-2-F6-IGFR	GFREGQFYDWFVAQVT	24.6	17.5	--	--	--
E4D-2-F7-IGFR	GFREGDFYQWFAAQVT	19.0	17.5	--	--	--
E4D-3-B7-IGFR	GFREGNFYDWFVAQVT	23.0	16.4	--	--	--
E4D-1B-C12-IGFR	GFRDGSFYDWFVAQVT	23.0	16.1	--	--	--
E4D-3-B1-IGFR	GFREGHFYEWFQAQVT	21.6	16.0	--	--	--
E4D-2-E2-IGFR	GFREGDFYDWFSAQVT	21.9	14.1	--	--	--
E4D-2-D1-IGFR	GFREGHFYDWFDAQVT	24.5	13.2	--	--	--
E4D-1-D4-IGFR	GFREGYFYDWFKAQVT	18.9	12.4	--	--	--
E4D-1B-A10-IGFR	GFREGHFYDWFEAQVT	23.9	10.8	--	--	--
E4D-1B-A3-IGFR	GFREGDFYDWFEAQVT	22.2	10.8	--	--	--
E4D-1-B5-IGFR	GFREGTFYDWFVAQVT	19.0	10.8	--	--	--

FIG. 1J-1

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4Dβ-4-F8-IR	GFREGSFYDWFAAQVT	39.3	35.6	44.4	0.8	1.2
E4Dβ-4-E12-IR	GFREGSFYEWFDAQVT	40.2	27.8	33.4	0.8	1.2
E4Dβ-4-H12-IR	GFREGAFYDWFEAQVT	41.2	27.1	32.3	0.8	1.2
E4Dβ-4-C9-IR	GFREGQFYDWFAAQVT	38.0	22.5	27.6	0.8	1.2
E4D□-4-H9-IR	GFREGNFYDWFAAQVT	38.7	33.3	36.6	0.9	1.1
E4D□-4-G9-IR	GFREGDFYDWFAAQVT	10.9	4.9	5.6	0.9	1.1
E4Dβ-4-F12-IR	GFREGSFYEWFEAQVT	14.8	5.9	6.1	1.0	1.0
E4Dβ-4-F9-IR	GFREGGFYDWFLAQVT	39.3	31.3	28.3	1.1	0.9
E4Dβ-4-F7-IR	GFREGGFYAWFAAQVT	31.0	22.2	19.5	1.1	0.9
E4Dβ-4-B7-IR	GFREGGFYEWF?AQVT	--	--	--	--	--

FIG. 11-3

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4Dα-4-H5-IR	GFREGSFYEWFQAQVT	47.2	36.0	14.7	2.4	0.4
E4Dα-1-B12-IR	GFREGNFYDWFAAQVT	47.6	33.4	13.8	2.4	0.4
E4Dα-4-G2-IR	GFREGSFYDWFVAQVT	23.4	20.4	8.6	2.4	0.4
E4Dα-3-F9-IR	GFREGDFYDWFVAQVT	36.2	15.6	6.3	2.5	0.4
E4Dα-4-G6-IR	GFREGDFYQWFVAQVT	26.0	4.9	2.0	2.5	0.4
E4Dα-4-H9-IR	GFREGGFYDWFVAQVT	47.8	24.8	9.5	2.6	0.4
E4Dα-2-C10-IR	GFREGDFYGWFQAQVT	42.4	23.2	9.0	2.6	0.4
E4Dα-1-B2-IR	GFREGVFYDWFVAQVT	39.4	18.7	7.2	2.6	0.4
E4Dα-3-F12-IR	GFREGGFYEWFQAQVT	38.9	16.6	5.6	3.0	0.3
E4Dα-2-D11-IR	GFREGSFYDWFQAQVT	40.2	11.1	3.3	3.4	0.3
E4Dα-4-H2-IR	GFREGNFYEWFQAQVT	37.8	33.9	8.2	4.1	0.2
E4Dβ-4-A12-IR	GFREGKFYDWFLAQVT	41.1	8.3	28.7	0.3	3.5
E4Dβ-4-A10-IR	GFREGEFYEWFVAQVT	5.8	1.2	2.4	0.5	2.0
E4Dβ-4-E10-IR	GFREGRFYDWFVAQVT	9.6	1.2	2.2	0.5	1.8
E4Dβ-4-B11-IR	GFREGTFYDWFVAQVT	36.1	15.2	26.9	0.6	1.8
E4Dβ-4-C10-IR	GFREGEFYEWFAAQVT	27.8	13.3	23.7	0.6	1.8
E4Dβ-4-E8-IR	GFREGDFYEWFEAQVT	28.7	16.7	28.2	0.6	1.7
E4Dβ-4-G7-IR	GFREGHFYDWF?AQVT	30.9	14.7	24.7	0.6	1.7
E4Dβ-4-C8-IR	GFREGEFYDWFVAQVT	35.5	22.5	32.9	0.7	1.5
E4Dβ-4-A8-IR	GFREGSFYDWFVAQVT	31.2	14.5	22.2	0.7	1.5
E4Dβ-4-A9-IR	GFREGSFYDWFGAQVT	35.8	9.0	13.1	0.7	1.5
E4Dβ-4-G11-IR	GFREGTFYDWFQAQVT	28.9	9.7	13.6	0.7	1.4
E4Dβ-4-B9-IR	GFREGNFYEWFTAQVT	27.2	9.1	12.5	0.7	1.4
E4Dβ-4-F10-IR	GFREGSFYNWFQAQVT	7.7	1.5	2.1	0.7	1.4
E4Dβ-4-D12-IR	GFREGNFYDWFVAQVT	41.1	27.2	36.1	0.8	1.3
E4Dβ-4-B8-IR	GFREGDFYDWFVAQVT	35.9	27.0	35.2	0.8	1.3
E4Dβ-4-G10-IR	GFREGAFYDWFAAQVT	38.5	25.5	33.7	0.8	1.3
E4Dβ-4-D9-IR	GFREGSFYDWFEAQVT	34.1	19.3	25.7	0.8	1.3

FIG. 1I-2

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4Dα-1-B8-IR	GFREGQRWYWFVAQVT	40.7	1.0	12.3	0.1	12.3
E4Dα-3-E5-IR	GFREGYFYDWFLAQVT	39.6	2.0	1.5	1.3	0.8
E4Dα-1-A1-IR	GFREGDFYEWFVAQVT	48.7	44.9	31.4	1.4	0.7
E4Dα-2-D9-IR	GFREGQFYEWFAAQVT	22.9	3.3	2.4	1.4	0.7
E4Dα-1-B3-IR	GFREGTFYDWFVAQVT	41.8	38.6	26.5	1.5	0.7
E4Dα-1-A6-IR	GFREGNFYDWFEAQVT	56.3	51.2	32.6	1.6	0.6
E4Dα-1-A10-IR	GFREGAFYDWFEAQVT	48.9	42.2	26.5	1.6	0.6
E4Dα-1-A8-IR	GFREGAFYDWFVAQVT	46.9	41.5	26.2	1.6	0.6
E4Dα-1-B1-IR	GFREGKFYQWFEAQVT	44.1	31.1	19.7	1.6	0.6
E4Dα-2-C9-IR	GFREGDFYDWFQAQVT	34.0	8.1	4.8	1.7	0.6
E4Dα-1-A3-IR	GFREGTFYEWFVAQVT	45.3	40.3	22.5	1.8	0.6
E4Dα-1-A9-IR	GFREGNFYDWFVAQVT	46.9	41.0	22.5	1.8	0.5
E4Dα-3-F3-IR	GFREGQFYEWFLAQVT	37.2	14.1	8.0	1.8	0.6
E4Dα-2-D3-IR	GFREGQFYDWFLAQVT	35.1	16.3	8.7	1.9	0.5
E4Dα-2-D6-IR	GFREGEFYDWFQAQVT	33.2	5.6	2.8	2.0	0.5
E4Dα-3-F10-IR	GFREGQFYDWFRAQVT	27.8	4.5	2.3	2.0	0.5
E4Dα-2-D5-IR	GFREGYFYEWFQAQVT	43.8	23.8	11.4	2.1	0.5
E4Dα-3-F4-IR	GFREGDFYQWFEAQVT	25.9	7.6	3.7	2.1	0.5
E4Dα-3-E3-IR	GFREGSFYGWFQAQVT	34.6	4.0	1.9	2.1	0.5
E4Dα-3-F8-IR	GFREGSFYAWFQAQVT	20.9	16.0	7.4	2.2	0.5
E4Dα-2-C1-IR	GFREGQFYDWFVAQVT	43.1	11.6	5.0	2.3	0.4
E4Dα-1-B4-IR	GFREGIFYEWFVAQVT	45.3	6.6	2.9	2.3	0.4

FIG. 1I-1

Clone Parental/Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19	4	--	--	--
A6L-4-F8-IGFR	YRGMMVQGRISDGAGKVASVSPVRIGQKVIAVNFYDWFV	26	28	--	--	--
A6L-2-G9-IGFR	YRGRLGLGRISDVAGKVACDPSARIGQKVLPVNFYDWFV	39	22	--	--	--
A6L-4-E7-IGFR	YRGMLVLGRISDGAGRVASEPQARIGQKVFAVNFYDWFV	23	22	--	--	--
A6L-4-G10-IGFR	QGGMLVPGRISDGAGKVASQPPARIGPKGFAGNFYDWFV	19	22	--	--	--
A6L-2-E9-IGFR	YRGMRVLGRISDGAGKVASEPPTHIGQKVFPVNFYDWFV	38	21	--	--	--
A6L-2-D6-IGFR	YRGMLVLGRISDGAGKVGSEPAARIGQKVFALNFYDWFV	34	21	--	--	--
A6L-3-H12-IGFR	YRGQGMVLGRISDGAGKVASEPPGRIGQKVFPVNFYDWFV	24	21	--	--	--
A6L-4-A7-IGFR	YRGMLGLGRITGGAGKVASEPPDRIGQHVFVDNFYDWFV	20	20	--	--	--
A6L-4-B8-IGFR	DGMLVLGRISDGAGNVASEAPARIGQKVFAVNFYDWFV	20	19	--	--	--
A6L-4-G7-IGFR	YRGMRVRGRISDGAGKAASDPRARIGQTVLDVNFYDWFV	19	19	--	--	--
A6L-2-D9-IGFR	YRGMWVLGRISYGAGKVAYEPPARMGQKGFAVNFYDWFV	38	18	--	--	--
A6L-4-F7-IGFR	YRGMLVGGRIAGGAGIVASEPPARIGQKVFAVNFYDWFV	18	18	--	--	--
A6L-4-E12-IGFR	YRGLLGLGGISDGAGKVASEPPARNGQKVFAVNFYDWFV	15	13	--	--	--
A6L-4-H7-IGFR	YRGMLGLGRISAGAGKVASGAPARIGQEDFAVNFYDWFV	14	13	--	--	--
A6L-4-H12-IGFR	YRGMLALGRISEGAGKVASEPPARIGQNVFAVNFYDWFV	13	12	--	--	--
A6L-2-A4-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVLAVNFYDWFV	17	4	--	--	--
A6L-3-D10-IGFR	YPGMLVPGRISDGAGEGATDPPPRIGQKVFAFNFYDWFV	16	4	--	--	--
A6L-2-F6-IGFR	YRGMLVPGRISDGAGKVAYEPPARIGQKIFAVNFYDWFV	15	4	--	--	--
A6L-2-B11-IGFR	YRGVLVLGRVSDGVGKVASEPPAHRGQRVFGVNFYDWFV	26	3	--	--	--
A6L-1-B7-IGFR	YRRMLVLGRISDGAANVASGPPDRIGQKVFAGNFYDWFV	23	3	--	--	--
A6L-1-D8-IGFR	YRRMLALGRFSDVTGDVASEPPAHIGQKVVAVNFYDWFV	23	3	--	--	--
A6L-0-A11-IGFR	YRGMVVRGRIFDGPGKVASEPRARIGQKVFAVNFYDWFV	19	3	--	--	--
A6L-3-B7-IGFR	YRGMLILGRISDGAGKVASEPPARVGQDVVAVNFYDWFV	9	3	--	--	--
A6L-1-G7-IGFR	YPGRLVGGRISDGVGKVASEPPGRIGQKVFAVNFYDWFV	20	2	--	--	--
A6L-1-B9-IGFR	QRGLLVLGRIFDGAGKVASDPPARIGQKDFADNFYDWFV	18	2	--	--	--
A6L-1-C9-IGFR	YRGMLVLGRISDGAGKVAFEPPARIGQNVFAVNFYDWFV	18	2	--	--	--
A6L-0-G10-IGFR	YRCMPVLGRISDGAG#VASDRPARIGQKVFAVNFYDWFV	18	2	--	--	--
A6L-1-G8-IGFR	YRGRLVLGRISDGAGKVAAEPPASMDSKVFAGNFYDWFV	15	2	--	--	--

FIG. 1H

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19.0	.4.0	--	--	--
A6L-4-H9-IR	YRAMLVLRRISDVAGIVDSEPPTRIGQKVFAGNFYDWFV	37.5	27.3	27.3	1.0	1.0
A6L-4-E1-IR	YRGMLVLGRISQGAGNVASEPSSRIGQKVFAGNFYDWFI	35.4	32.6	31.4	1.0	1.0
A6L-3-A5-IR	YRGMLVLGRISDGAGKVDYEPPARIGQKVFAGNFYDWFV	38.3	34.6	35.5	1.0	1.0
A6L-4-G4-IR	YRGMLGLGGISAGAGIVASEPPARVGQKVFAGNFYDWFV	30.4	17.7	15.2	1.2	0.9
A6L-4-H2-IR	YRGILFQGRIPDGAGKVASEPPTRIGERVFAVNFYDWFV	36.1	4.2	3.6	1.1	0.9
A6L-4-E6-IR	QGGMPVLGRISDGAGKVAFEPPARIGQKVFAGNFYDWFV	28.6	24.1	22.7	1.1	0.9
A6L-4-H5-IR	YRGMLVLGRIQDGAGKVASEPPARIGQKVFTGNFYDWFV	37.2	24.6	23.1	1.1	0.9
A6L-4-H3-IR	QRGMLVLGGVSDGAGKVASDPPASIGQNVFAVNFYDWFV	37.1	9.1	7.2	1.3	0.8
A6L-4-E5-IR	YPGMLILDRISDGASKVVSEPPASIGQKVFAVNFYDWFV	42.1	30.6	24.4	1.3	0.8
A6L-3-C5-IR	YRGMLVLDRISDGAGKVASEQPARIGQEVYAVNFYDWFV	42.2	21.9	17.5	1.2	0.8
A6L-4-G6-IR	YRGMLDLGRISGGVGKVASESPARIGQKVYAVNFYDWFV	29.8	4.3	2.8	1.5	0.7
A6L-3-D4-IR	QRGMMVLGRISDGAGEVASEKVFAVNFYDWFV	39.9	12.4	8.4	1.5	0.7
A6L-3-A7-IR	QRGMLVLGRVSDGAGKVDSAPPARIGQKVFAGNFYDWFV	31.0	21.2	14.0	1.5	0.7
A6L-3-A6-IR	QRGMLVLGRMSDGAGKVAFEPPARIGQRGFAGNFYDWFV	25.5	12.3	8.8	1.4	0.7
A6L-4-E7-IR	QRGTLVLGRISDGAGKAASEPPARIGQNVFAVNFYDWFV	38.4	12.5	7.1	1.7	0.6
A6L-3-C6-IR	QRGMLVLDRISDGAGKVAAEPPARIGQKVFALNFYDWFI	28.8	10.9	6.7	1.6	0.6
A6L-4-F5-IR	QRGMLVLGRISDGAGEVASEPPARIGEKVYAVNFYDWFV	33.8	6.3	4.1	1.5	0.6
A6L-3-B7-IR	QRGILVRGRISDGAGKVGSEPPARSGEKVFAVNFYDWFI	27.6	9.4	5.0	1.9	0.5
A6L-4-F4-IR	QLGMVVLGRISDGSGKAASEPAARISQKVFAVNFYDWFV	38.9	17.6	9.4	1.9	0.5
A6L-4-E3-IR	QRGMLVLGRISDGDGKVASEPPARIGQRVFAVNFYDWFV	38.0	6.9	3.8	1.8	0.5
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1

FIG. 1G-2

Cl ne Parental/Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19.0	.4.0	--	--	--
A6L-3-D1-IR	QRGMLVRGRISHGAGKIAYEPPDCLGQKACAVNFYDWFV	22.6	19.8	26.5	0.7	1.3
A6L-4-H7-IR	QRGMLLLGRISDDAGKVASEPSARRGQKVFAFNFYDWFV	37.5	3.5	4.2	0.8	1.2
A6L-4-H4-IR	YRGILVLGRISEGAGKVASEPAARIGQKVFADFYDWFV	38.5	21.1	25.8	0.8	1.2
A6L-4-E4-IR	QRGMLALGRISDGAGKVASEPPAGIGQKVFAFNFYDWFV	38.1	5.4	6.0	0.9	1.1
A6L-4-G7-IR	FRGRLVLGHFSDGAGKVGSEPAARIGQKVFDVNFYDWFV	38.6	16.2	18.5	0.9	1.1
A6L-3-C3-IR	YRGMLVLGRISDGAGKVASEPPARIGQEVFADNFYDWFV	34.7	21.8	23.1	0.9	1.1
A6L-3-B6-IR	YRGMLVLGRISDGAGEVASEPPARIGQEVFALNFYDWFV	33.1	27.8	30.3	0.9	1.1
A6L-4-G11-IR	VPWYAGSGSSSDGAGKVASEPPARIDQKVFAVNFYDWFV	27.6	2.0	2.0	1.0	1.0
A6L-4-G12-IR	YRGQLVLGRISYGAGKVGCDPPARIGQKDWAVNFYDWFV	32.0	2.3	2.3	1.0	1.0
A6L-3-A10-IR	QRGLLVLGRFSDGAGNVASEPPAGIGQEVFPVNFYDWFV	21.1	2.4	2.4	1.0	1.0
A6L-4-E12-IR	QRGMLVLGRISDGAGKVAAEPPDCLGQKVCAVNFYDWFV	3.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR	QRGMRVLGRISDGAGKVASELPPRIGQKDFAVNFYDWFV	30.1	3.8	3.8	1.0	1.0
A6L-4-G8-IR	QRGMLVLGSISDGAGKVAYEAPARIGQTVFAVNFYDWFV	37.9	4.7	4.7	1.0	1.0
A6L-3-C12-IR	QPWCAGSGRIYDGACKVASEPPAHIGQEVFAVNFYDWFV	29.5	5.7	5.7	1.0	1.0
A6L-4-H11-IR	QRGMLVLDRISDGAGKVASGPPARIGQNVLAVNFYDWFV	35.4	9.6	9.6	1.0	1.0
A6L-4-F10-IR	YRGMLVVGRISDGTGKVASQPPARIGQKVFAVNFYDWFV	31.6	10.5	10.5	1.0	1.0
A6L-4-E9-IR	YRGMLVLGRISDGAGKVASVPPAHIGQKVFAFNFYDWFV	39.8	12.9	12.9	1.0	1.0
A6L-4-H8-IR	QHGMLVLGRVSVGAGKVPSEPQARIGHKVFDVNFYDWFV	38.2	14.6	14.6	1.0	1.0
A6L-3-A11-IR	YSGYAGSGSFSDGAGKVASEPPARISQEVLADNFYDWFV	29.0	17.5	17.5	1.0	1.0
A6L-4-F9-IR	YRGMLVLGRISDGAGKVASEPPARIGQKVSAVNFYDWFV	35.7	18.4	18.4	1.0	1.0
A6L-4-G2-IR	YHGKLDLGRISVGVGKVASEPPARIGQKVFADNFYDWFV	29.5	21.4	20.7	1.0	1.0
A6L-4-E8-IR	YRGQAGSGVGSLTVAGKVASDPPARIGQKVFADNFYDWFV	28.7	21.6	21.6	1.0	1.0
A6L-4-H10-IR	HRGMLVLGRISEGAGNVDPEPPARIGQNVFAGNFYDWFV	30.0	22.1	22.1	1.0	1.0
A6L-4-G9-IR	QRGMPVLGRISDGAGKVGSEPPARIARKVFPVNFYDWFI	37.1	22.6	22.6	1.0	1.0
A6L-4-F7-IR	QGGLLVTGRISDGAGKVASEPPGGIGQKVFAGNFYDWFV	28.6	23.6	24.4	1.0	1.0
A6L-4-E11-IR	YPWYGGSGTYLDGAGKVASEPPARIDQQVFAGNFYDWFV	38.4	26.5	26.5	1.0	1.0

FIG. 1G-1

Ratios over Background		Comparisons				
Clone	Sequence	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-1-D5-IGFR	RVGSGMEDLGNFYDWFVRQAQ	25	5	--	--	--
A6S-1-A2-IGFR	KDPVTVSQGRNFYDWFVVQIQ	20	5	--	--	--
A6S-3-E6-IGFR	DARDHGVWVMSNFYDWFVAQVS	20	5	--	--	--
A6S-1-G3-IGFR	VATVHVGGGMNFYDWFVAQVG	19	5	--	--	--
A6S-3-G4-IGFR	CADPGACSSLNFYDWFVQMRG	21	4	--	--	--
A6S-3-H8-IGFR	NPTSVQQYGVNFYDWFVNVLS	20	4	--	--	--
A6S-3-E3-IGFR	RPSLPEVRPGNFYDWFVQSVR	19	4	--	--	--
A6S-3-D9-IGFR	SLQGADFQQGNFYDWFVSELA	17	4	--	--	--
A6S-2-A1-IGFR	LSSRGRVTMRNFYDWFVAQVV	31	3	--	--	--
A6S-1-H4-IGFR	HKSWTTMSPLNFYDWFVAQVE	18	3	--	--	--
A6S-3-C1-IGFR	RPVIGGGGTRNFYDWFVAQMI	17	3	--	--	--
A6S-3-B10-IGFR	YDQDPPYWGLNFYDWFVREVA	16	3	--	--	--

FIG. 1F-3

Rati s over Background Clone Design	Sequence XXXXXXXXXXXNFYDWFVXXXX	Comparisons E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
A6S-4-A6-IGFR	HVEHMAVGDGNFYDWFVVQLR	21	23	--	--	--
A6S-4-E3-IGFR	RGMTGMVGRGNFYDWFVGQLR	21	23	--	--	--
A6S-4-D3-IGFR	GLRSEQGNRLNFYDWFVAQIA	20	23	--	--	--
A6S-3-E10-IGFR	RVREKLPRPENFYDWFVNQIH	23	22	--	--	--
A6S-4-D1-IGFR	SNPSRQDASVNFYDWFVREVA	22	22	--	--	--
A6S-4-B2-IGFR	QSVDLSRPDSNFYDWFVEVLS	21	22	--	--	--
A6S-4-A2-IGFR	IGGQGQHQDGNFYDWFVEALA	20	22	--	--	--
A6S-4-A5-IGFR	VEVQRHIRKDNFYDWFVKQID	19	22	--	--	--
A6S-4-C1-IGFR	CWARPCGDAANFYDWFVQQAS	16	22	--	--	--
A6S-4-B1-IGFR	RHERGKEGPGNFYDWFVSQVV	19	21	--	--	--
A6S-4-B4-IGFR	ERSPRPALASNFYDWFVQQVV	19	21	--	--	--
A6S-4-D4-IGFR	IARMRETFQPNFYDWFVDQLA	18	21	--	--	--
A6S-3-F8-IGFR	GRGQGLKRPDNFYDWFVAAAK	25	20	--	--	--
A6S-3-H9-IGFR	YSIEVQDWNENFYDWFVSQLG	23	20	--	--	--
A6S-3-G2-IGFR	TWMWEERKQDNFYDWFVGQLK	21	20	--	--	--
A6S-4-H2-IGFR	VTFTSAVFHENFYDWFVRQVS	19	20	--	--	--
A6S-4-A3-IGFR	LAINDLVTHKNFYDWFVDQLR	18	20	--	--	--
A6S-3-G10-IGFR	GAVGLAEAGPNFYDWFVSQVQ	24	19	--	--	--
A6S-3-E5-IGFR	RYRGERHDGRNFYDWFVEQVN	21	19	--	--	--
A6S-3-H2-IGFR	QGAEGRLSEGNFYDWFVQAVS	21	19	--	--	--
A6S-3-G3-IGFR	PRLHMGSDMGDFYDWFVVQIA	21	18	--	--	--
A6S-4-H1-IGFR	IVAGARHSEVNFYDWFVIQVR	18	18	--	--	--
A6S-4-G1-IGFR	AELVGAGVRGNFYDWFVDQLV	16	16	--	--	--
A6S-4-A1-IGFR	DSSRLWLGERNFYDWFVAQIS	17	12	--	--	--
A6S-2-F1-IGFR	VGQVGRYVRSNFYDWFVQQAM	30	8	--	--	--
A6S-2-G1-IGFR	RPQLVESGSKNFYDWFVQVVR	30	8	--	--	--
A6S-1-C5-IGFR	RIHNQTERGGNFYDWFVHQLV	27	7	--	--	--
A6S-2-B2-IGFR	EMYGDTSERVNFYDWFVSALQ	30	5	--	--	--

FIG. 1F-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D sign	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-4-E4-IGFR	ERSAAGFREGNFYDWFVAQVN	27	32	--	--	--
A6S-2-D2-IGFR	RAERGSMRDSNFYDWFVQQLP	36	30	--	--	--
A6S-2-F2-IGFR	LAMSVASRPANFYDWFVAQIV	35	30	--	--	--
A6S-4-F3-IGFR	HNSSSPMRTGNFYDWFVQELR	26	30	--	--	--
A6S-4-G4-IGFR	SALSGPVQPINFYDWFVTGM	26	30	--	--	--
A6S-4-G3-IGFR	GAQAIREIHHNFYDWFVAQVT	21	29	--	--	--
A6S-2-H2-IGFR	RGQRESDSGTNFYDWFVGAIR	40	28	--	--	--
A6S-2-E3-IGFR	VQEGLSGMEGNFYDWFVDQLF	36	28	--	--	--
A6S-4-C6-IGFR	RLDRSSTSGVNFYDWFVAQVG	25	28	--	--	--
A6S-4-F5-IGFR	GSQHSGREPHNFYDWFVAQVG	24	28	--	--	--
A6S-4-H3-IGFR	GRGDQRHETTNFYDWFVRELQ	20	28	--	--	--
A6S-4-H4-IGFR	PRMVEKPSEDNFYDWFVTQLS	20	28	--	--	--
A6S-2-H1-IGFR	RVGIQVDPHTNFYDWFVIQLT	42	27	--	--	--
A6S-4-E6-IGFR	RSSGGLLSQGNFYDWFVSQLE	24	26	--	--	--
A6S-4-B6-IGFR	SDARQAGLQENFYDWFVSQVR	23	26	--	--	--
A6S-4-D2-IGFR	PPYRSSRLGENFYDWFVMQVR	19	26	--	--	--
A6S-4-G5-IGFR	QEVTRTRDDKNFYDWFVSQIF	18	26	--	--	--
A6S-2-A3-IGFR	SRAPYGSTAGNFYDWFVQAVS	37	25	--	--	--
A6S-4-E2-IGFR	?DGQSVSSKGNFYDWFVQQMT	25	25	--	--	--
A6S-4-G6-IGFR	RLMGGIAEPQNFYDWFVREVA	20	25	--	--	--
A6S-4-G2-IGFR	SAGHHMPRESNFYDWFVDQVV	25	24	--	--	--
A6S-4-D6-IGFR	LGAAETWDGINFYDWFVKQVS	22	24	--	--	--
A6S-4-F4-IGFR	VGHSGVPPYPNFYDWFVMQVS	22	24	--	--	--
A6S-4-C3-IGFR	VTMLDKGAQDNFYDWFVREVA	21	24	--	--	--
A6S-4-H5-IGFR	HHSPGNEHGYNFYDWFVLQVA	19	24	--	--	--
A6S-4-H6-IGFR	GSIAQLIMRANFYDWFVEQTN	18	24	--	--	--
A6S-4-F6-IGFR	LKGSSQPLSVNFYDWFVQQIK	17	24	--	--	--
A6S-3-H1-IGFR	PASNKNSLAENFYDWFVQQTR	30	23	--	--	--

FIG. 1F-1

Clone Design	Sequence XXXXXXXXXXXNFYDWFVXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
A6S-4-G1-IR	NGVERAGTGDNFYDWFVAQLH	36.2	31.8	15.7	2.0	0.5
A6S-1-A3-IR	PFAGKGDKTGNFYDWFVSLTG	39.9	12.6	6.0	2.1	0.5
A6S-3-F12-IR	GMPQEYMDQVNFYDWFVAQVD	41.4	7.4	4.0	1.9	0.5
A6S-4-G2-IR	MGTPAVGDGANFYDWFVRQLG	26.7	7.0	3.5	2.0	0.5
A6S-1-B1-IR	SKCKAWYGANNFYDWFVWQVD	30.6	3.7	1.9	1.9	0.5
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4

FIG. 1E-3

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-3-E9-IR	TTCHPRGEDCNFYDWFVLQLR	36.6	9.0	8.9	1.0	1.0
A6S-3-E1-IR	VRGNDSVLRANFYDWFVDQLS	36.7	6.8	6.9	1.0	1.0
A6S-4-H12-IR	TPRSQVRSDHNFYDWFVYQLA	46.3	6.1	5.8	1.1	1.0
A6S-2-D3-IR	ESLTGSRPDRNFYDWFVQQTS	37.0	5.3	5.1	1.0	1.0
A6S-3-E8-IR	PQSLTEVRTGNFYDWFVVQLH	42.7	5.2	5.1	1.0	1.0
A6S-1-A12-IR	DVGMGRVKETNFYDWFVRQLI	39.7	2.1	2.1	1.0	1.0
A6S-4-H3-IR	GADDIRSLNTNFYDWFVNQLS	18.6	3.1	2.9	1.1	0.9
A6S-3-F7-IR	GVSIQAGYKTNFYDWFVEAVR	46.2	2.3	2.1	1.1	0.9
A6S-2-D8-IR	VGEHRQMSVGNFYDWFVMQIA	31.2	2.0	1.7	1.2	0.9
A6S-3-F10-IR	GSSLGRSGPGNFYDWFVDQLE	39.0	5.9	4.5	1.3	0.8
A6S-4-G11-IR	HRQQDVVRQGNFYDWFVQALE	44.8	4.3	3.3	1.3	0.8
A6S-2-D2-IR	QDTFLTAREGNFYDWFIRALE	33.5	3.6	2.7	1.3	0.8
A6S-4-G8-IR	EAIMREEGQANFYDWFVRQLE	11.1	2.5	1.9	1.3	0.8
A6S-4-H6-IR	VCDVSTGGGTNFYDWFVCQVG	22.4	2.4	1.9	1.3	0.8
A6S-2-D10-IR	PQPRSASTPLNFYDWFVQATG	41.3	2.1	1.7	1.2	0.8
A6S-3-F4-IR	GVSRGSGGDPNFYDWFVMQLR	37.0	13.5	9.9	1.4	0.7
A6S-4-G9-IR	GPGRHDSSRGNFYDWFVEQLA	36.2	11.8	7.8	1.5	0.7
A6S-3-F5-IR	ERFALEVQGSNFYDWFVRQVI	48.1	7.2	4.8	1.5	0.7
A6S-4-H1-IR	NLKSSATVGGNFYDWFVEQL	18.3	3.6	2.6	1.4	0.7
A6S-3-F6-IR	MEGPPAGGPLNFYDWFVAQVD	18.7	2.9	1.9	1.5	0.7
A6S-3-F11-IR	RLDVAGHRGGNFYDWFVKQLH	33.8	2.0	1.4	1.4	0.7
A6S-2-C6-IR	PWSDHEALNQNFYDWFVSQVL	46.7	19.2	12.1	1.6	0.6
A6S-4-G4-IR	EDRLGNGESTNFYDWFVRQLA	36.9	18.2	10.7	1.7	0.6
A6S-4-G12-IR	GKLVASTLDDNFYDWFVRQLS	32.8	12.8	7.9	1.6	0.6
A6S-2-D7-IR	SGPVVQTQNGNFYDWFVHQLR	33.2	12.0	7.1	1.7	0.6
A6S-4-G10-IR	VDRAGPAGSDNFYDWFVAQLD	33.9	10.8	6.8	1.6	0.6
A6S-3-F9-IR	SLGRNDRPDENFYDWFVSQVQ	44.3	9.6	5.7	1.7	0.6
A6S-3-F2-IR	RVMATANAPMNFYDWFVVQLQ	23.2	4.3	2.5	1.7	0.6

FIG. 1E-2

Clone Design	Sequence XXXXXXXXXXXNFYDWFVXXXX	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
A6S-3-E12-IR	GRVDWLQRNANFYDWFVAELG	26.2	1.3	8.0	0.2	6.2
A6S-2-C1-IR	RMYFSTGAPQNFYDWFVQEWD	41.2	1.3	7.0	0.2	5.4
A6S-1-A7-IR	HHTQGLQVQRNFYDWFVNELR	47.2	2.3	11.1	0.2	4.8
A6S-2-C8-IR	MHRMQHDGTSNFYDWFVLQWA	44.9	1.5	5.5	0.3	3.7
A6S-3-E10-IR	AMHVVAQGGPNFYDWFVRELR	46.9	1.6	5.0	0.3	3.1
A6S-2-D5-IR	AIQMNGNLAFNFYDWFVRELT	31.9	1.2	3.7	0.3	3.1
A6S-1-B2-IR	TDRKSVQEPRNFYDWFVWAAR	31.6	1.8	5.3	0.3	2.9
A6S-1-A4-IR	PHGHRGFAQSNFYDWFVTQEE	43.3	3.6	9.2	0.4	2.6
A6S-4-G3-IR	RLASASVPGQNFYDWFVDQLL	31.3	2.3	5.1	0.5	2.2
A6S-4-H8-IR	RQSEFSTLNSNFYDWFVRELE	11.5	1.7	3.6	0.5	2.1
A6S-3-E11-IR	GQAQLSIRDVNFYDWFVQQIV	26.3	2.3	4.4	0.5	1.9
A6S-1-A1-IR	MSEPAVGVNGNFYDWFVAQLF	36.9	3.7	6.5	0.6	1.8
A6S-2-C9-IR	VGTGRARLDRNFYDWFVGQYS	43.6	1.3	2.3	0.6	1.8
A6S-2-C4-IR	SREAVQKRNANFYDWFVQQLS	34.5	5.6	9.6	0.6	1.7
A6S-4-H10-IR	LAQFAGSRNQNFYDWFVEQLG	39.2	4.4	6.9	0.6	1.6
A6S-4-G7-IR	GQEYFDQMGLNFYDWFVRELD	19.1	1.4	2.2	0.6	1.6
A6S-4-H2-IR	RQPSQPPHGSNFYDWFVEAIN	25.5	2.6	3.9	0.7	1.5
A6S-2-C3-IR	LMQSLGSGSTNFYDWFVQQMV	31.1	1.6	2.4	0.7	1.5
A6S-2-C11-IR	DQQRSACDGTNFYDWFVCQLS	20.9	3.3	4.6	0.7	1.4
A6S-3-F3-IR	LDGTKACQRVNFYDWFVCQTE	37.1	3.0	4.2	0.7	1.4
A6S-3-E5-IR	PEARRTVVHSNFYDWFVAQLS	31.6	2.5	3.5	0.7	1.4
A6S-1-B7-IR	PWMLSVGIQDNFYDWFVGLDS	49.2	1.6	2.3	0.7	1.4
A6S-3-E7-IR	ASHQRGGSSDNFYDWFVAQMR	37.2	5.0	6.3	0.8	1.3
A6S-4-G6-IR	TLEREGEFSGNFYDWFVEQLH	16.8	3.1	4.0	0.8	1.3
A6S-2-C2-IR	DRQSIGSVHGDFYDWFVSALG	29.7	2.4	3.1	0.8	1.3
A6S-3-F1-IR	DWDKLGSLSENFYDWFVDQLA	29.7	2.3	3.0	0.8	1.3
A6S-2-C5-IR	VRVVLNQSGRNFYDWFVIQLE	42.9	6.1	7.0	0.9	1.1
A6S-3-E4-IR	MASWQSRTPDNFYDWFVRELS	20.9	2.1	2.3	0.9	1.1

FIG. 1E-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20-4-B9-IGFR	DPERMQSDVGFYEWFRAAVG	40.1	16.6	--	--	--
R20-4-F8-IGFR	DIGSDGHGRRWDSFYRWFEM	39.2	13.9	--	--	--
R20-4-G12-IGFR	PFYQWFLDQSVGGSRGGGLR	36.7	8.0	--	--	--
R20-4-D10-IGFR	AVAPLSVRGRDSGFYSWFSS	40.2	4.1	--	--	--

FIG. 1D

Cl ne Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20α-3-20D3-IR	IGGQGQHQDGNFYDWFVEALA	46.3	36.2	7.0	5.2	0.2
R20α-3-20F1-IR	VFWNCRSQQLDFYEWFEQAA	49.0	26.0	2.8	9.3	0.1
R20α-3-20H1-IR	RVAGAISAPGLVSNKQDGLFYSWFRE	45.6	35.3	3.3	10.7	0.1
R20α-3-20D1-IR	VLQARHGCDSVSDCFYEWFA	50.8	37.5	3.0	12.5	0.1
R20β-4-B12-IR	GAFYRWFHEALVGSERVPDV	41.9	2.9	5.7	0.5	2.0
R20β-4-H3-IR	HEAFYDWFSALVDGGYELMG	13.9	5.8	2.4	2.4	0.4
R20β-4-D10-2-IR	RIGGGWARSEGFYEWFVREL	21.5	7.3	2.9	2.5	0.4
R20β-4-C8-IR	LPAGGA?GFA?RGFYEWFES	44.9	31.1	9.6	3.2	0.3
R20β-4-E7-IR	GHSWALVRHVDRLFYEWFDL	45.0	18.8	5.9	3.2	0.3
R20β-4-E7-2-IR	LGTSAGQGVGHRAFYQWFQS	45.0	18.8	5.9	3.2	0.3
R20β-4-G3-IR	RGGGTFYEWFESALRKHGAG	38.6	7.5	2.0	3.8	0.3
R20β-4-H6-IR	NSSGQQVVGLTFYSWFASQV	14.8	7.6	2.0	3.8	0.3
R20β-4-G11-IR	FYGWFSRQLSLTPRDDWGLP	39.4	7.5	1.9	3.9	0.3
R20β-4-G8-IR	RMFYEWFWSQMGAGPTEGSA	41.2	15.1	3.4	4.4	0.2
R20β-4-H9-IR	IGGQGQHQDGNFYDWFVEALA	43.1	8.8	2.0	4.4	0.2
R20β-4-H8-IR	RDKPTDQEEQNWSFYEWFRH	47.9	43.7	9.3	4.7	0.2
R20β-4-B8-IR	WSALLSVMDTGFYAWFDDAV	44.0	40.1	8.4	4.8	0.2
R20β-4-E2-IR	SRDQTNFTFNSAGFYGWFER	16.3	13.9	2.4	5.8	0.2
R20β-4-F4-IR	GVGTLTMSSDAFYTWFV	15.3	5.9	1.0	5.9	0.2
R20β-4-A8-IR	IGGSFVEFYGWFNDQV	43.3	36.0	6.0	6.0	0.2
R20β-4-C4-IR	DIGSDGHGRRWDSFYRWFEM	17.3	26.8	4.3	6.2	0.2
R20β-4-D7-IR	VLQARHGCDSVSDCFYEWFA	44.8	36.2	5.6	6.5	0.2
R20β-4-D2-IR	DPERMQSDVGFYEWFRAAVG	31.2	29.4	2.9	10.1	0.1

FIG. 1C

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-40B2-IR	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID	40.3	9.0	2.0	4.5	0.2
R40-4-40B12-IR	RMGLQALAHYRKSAGPIFLSSGSVIKGSEGDPFYAWFRLQ	60.4	12.9	2.0	6.5	0.2
R40-4-40G11-IR	MPVSLFRRVWDYRDGEHETLESHYVVPQAALDRLFYSWFS	52.6	37.5	2.0	18.8	0.1

FIG. 1A

Clon	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-D5-IGFR	PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM	--	--	--	--	--
R40-3-A6-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	--	--	--	--	--
R40-X-R35-IGFR	SGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	--	--	--	--	--

FIG. 1B

260. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 7.

261. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 8.

262. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 9.

263. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 10.

251. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 2 of IGF-1R.

252. An amino acid sequence comprising the sequence $WX_{123}GYX_{124}WX_{125}X_{126}$ wherein X_{123} is proline, glycine, serine, arginine, alanine or leucine, X_{124} is any amino acid; X_{125} is a hydrophobic amino acid; and X_{126} is any amino acid.

253. The amino acid sequence according to claim 252 wherein X_{123} is proline and X_{125} is leucine or phenylalanine.

254. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 1.

255. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 2.

256. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 3.

257. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 4.

258. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 5.

259. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 6.

245. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 1 of IR.

246. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 2 of IR.

247. A method of screening for a compound which binds to IGF-1R comprising:

i) immobilizing IGF-1R, or a fragment thereof, on a surface;

ii) incubating the IGF-1R, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-9, or an amino acid sequence selected from Figure 10, which binds IGF-1R and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind to IGF-1R;

iii) measuring the amount of labeled amino acid sequence bound to IGF-1R;

iv) determining from the amount of bound labeled peptide whether the compound has competitively bound to IGF-1R.

248. An amino acid sequence capable of bind to Site 1 or Site 2 of IGF-1R identified by the method according to claim 247, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.

249. The amino acid sequence according to claim 248 wherein the amino acid sequence is an IGF agonist.

250. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 1 of IGF-1R.

239. A method of treating a patient with an IGF sensitive tumor comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which is an IGF-1R antagonis, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.

240. The method according to claim 239 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.

241. The method according to claim 239 wherein the amino acid sequence is administered to the individual as a polypeptide.

242. A method of screening for a compound which binds to IR comprising:

i) immobilizing IR, or a fragment thereof, on a surface;

ii) incubating the IR, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-10, or an amino acid sequence selected from Figures 10-11, which binds IR and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind IR;

iii) measuring the amount of labeled amino acid sequence bound to IR;

iv) determining from the amount of bound labeled peptide whether the compound has competitively bound to IR.

243. An amino acid sequence capable of binding to Site 1 or Site 2 of IR identified by the method according to claim 242, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.

244. The amino acid sequence according to claim 243 wherein the amino acid sequence is an IR agonist.

231. The composition according to claim 230, wherein the peptide comprises the amino acid sequence NFYDWFV.

232. The pharmaceutical composition according to claim 230, wherein the peptide comprises the amino acid sequence QMKDIFYSLLASLAA.

233. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IR receptor at Site 1and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof, and a pharmaceutically acceptable carrier.

234. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYDWF.

235. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYSLLASL.

236. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which binds IR at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.

237. The method according to claim 236 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.

238. The method according to claim 236 wherein the amino acid sequence is administered to the individual as a polypeptide.

NSFYEALRMLSS,
SLNFYDALQLLA,
SSNFYQALMLLS,
SDGFYNAIELLS,
HETFYSMIRSLA,
HDPFYSMMKSLL and
WSDFYSYFQGL.

224. A kit for identifying a compound which binds IGF-1 receptor, comprising a IGF-1 receptor and an amino acid sequence selected from Formulas 1-10, or the amino acid sequences of Figures 9-11, which bind to the receptor at Site 1 or Site 2.

225. The kit according to claim 224, wherein the amino acid sequence comprises the amino acid sequence FYDWF.

226. The kit according to claim 225, wherein the amino acid sequence comprises the amino acid sequence SAKNFYDWFVKK.

227. The kit according to claim 226 wherein the amino acid sequence comprises the amino acid sequence FYSLLASL.

228. The kit according to claim 227 wherein the amino acid sequence comprises the amino acid sequence QMKDIFYSLLASLAAKK.

229. A kit for identifying a compound which binds IR comprising IR and an amino acid sequence selected from Formulas 1-10 or the amino acid sequences of Figures 9 and 11 which bind IR at Site 1 or Site 2.

230. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IGF-1 receptor at Site 1 and is an IGF agonist, with the proviso that the amino acid sequence is not IGF-1, insulin, or fragments thereof, and a pharmaceutically acceptable carrier.

AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENF YDWFVRQVSAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAAG APVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAA AGAPVPYPDPLEPRAA and

AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENFYDWFVRQV SAAAGAPVPYPDPLEPRAA.

220. A nucleic acid sequence encoding amino acid sequence which binds to IR at Site 1 and/or Site 2, with the proviso that the sequence is not insulin, IGF, or fragments thereof.

221. The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of FYDWF, FYEWF, FHENFYDWF, FHENFYDWFVRQVSK, DYKDVTFTSAVFHENFYDWFVRQVSKK,GRVDWLQRNANFYDWFV AELG and APTFYAWFNQQT.

222. The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of DYKDFYDAIDQLVRGSARAGGTRDKK and KDRAFYNGLRDLVGAVYGAWDKK.

223. The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of SFYEAIHQLLGV,

215. The amino acid sequence according to claim 203 wherein the two amino acid sequences are connected by a peptide or non-peptide linker.

216. The amino acid sequence according to claim 215 wherein the linker is a peptide consisting of about 2 to about 16 amino acids.

217. The amino acid sequence according to claim 215 wherein the linker is a non-peptide.

218. The amino acid sequence according to claim 217 wherein the linker is dialdehyde.

219. The amino acid sequence according to claim 203 wherein the amino acid sequence is selected from the group consisting of

DYKDDDDKFHENFYDWFVRQVSGSGSGLDALDRLMRYGEERPSLA AAGAP,

DYKDDDDKFHENFYDWFVRQVSGGSHLCVLEELFWGASLFGYCSG AAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSHLCVLEELFWGASL FGYCSGAAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWG ASLFGYCSGAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVP YPDPLEPRAA,

212. The amino acid sequence according to claim 211 wherein at least one of the sequences comprises LX_{15}, X_{16}, $LLX_{19}YF$.

213. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22}, X_{25}, X_{26}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{36}, X_{37}, X_{38}, X_{40}, and X_{41} are any amino acid, X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid, and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40}; X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} $X_{56}X_{57}X_{58}X_{59}$ X_{60} X_{61} wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60} and X_{61} are any amino acid; X_{43}, X_{46}, X_{49}, X_{50} and X_{54} are hydrophobic amino acids; X_{47} and X_{59} are cysteine; X_{48} is a polar amino acid; and X_{51} ,X_{52} and X_{57} are small amino acids; or X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid; X_{63}, X_{70}, and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

214. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88}, X_{89}, X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids or $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and wherein at least one of X_{104}, X_{105}, X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

206. The amino acid sequence according to claim 203, wherein at least one of the sequences is selected from the group consisting of $X_1X_2X_3X_4X_5$ wherein X_1, X_2, X_4, and X_5 are aromatic amino acids, and X_3 may be any polar amino acid; $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8, X_9, X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids; and $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

207. The amino acid sequence according to claim 206, wherein at least one of the sequences is $X_1X_2X_3X_4X_5$ wherein X_1, X_2, X_4, and X_5 are aromatic amino acids, and X_3 may be any polar amino acid.

208. The amino acid sequence according to claim 206 wherein at least one of the sequences comprises FYX_3WF.

209. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8, X_9, X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.

210. The amino acid sequence according to claim 209, wherein at least one of the sequences comprises $FYX_8X_9LX_{11}X_{12}L$.

211. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

198. The amino acid sequence according to claim 197 comprising ACVWPTYWNCG.

199. A method of providing insulin agonist activity to mammalian cells, said method comprising administering to said cells an amino acid sequence comprising DYKDLCQSWGVRIGWLAGLCPKK.

200. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figures 9 through 11.

201. An amino acid sequence comprising DYKDLCQSWGVRIGWLAGLCPKK.

202. An amino acid sequence comprising an amino acid sequence selected from the group listed in Figures 9 through 11.

203. An amino acid sequence comprising at least two amino acid sequences which independently bind IR, with the proviso that at least one of the sequences is not insulin or a fragment thereof.

204. The amino acid sequence according to claim 203 wherein the two amino acid sequences bind to Site 1 of IR.

205. The amino acid sequence according to claim 203 wherein one amino acid sequence binds to Site 1, and the other binds to Site 2 of IR.

188. The amino acid sequence according to claim 187 wherein at least two of the amino acids of X_{106} through X_{111} are tryptophan which are separated from each other by at least two amino acids.

189. The amino acid sequence according to claim 188 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.

190. The amino acid sequence according to claim 189 wherein the amino acid sequence comprises WPTYW.

191. The amino acid sequence according to claim 190 wherein X_{105} and X_{113} are cysteine residues.

192. The amino acid sequence according to claim 190 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.

193. The amino acid sequence according to claim 190 wherein X_{104} is alanine and X_{114} is glycine.

194. The amino acid sequence according to claim 193 wherein X_{105} is valine.

195. The amino acid sequence according to claim 194 wherein X_{112} is asparagine.

196. The amino acid sequence according to claim 202 wherein the affinity (K_d) of binding to IR is at least about 10^{-5} M.

197. An amino acid sequence which binds IR from mammalian cells comprising an amino acid sequence selected from the group listed in Figure 8.

179. The method according to claim 178 wherein X_{105} and X_{113} are cysteine residues.

180. The method according to claim 178 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.

181. The method according to claim 180 wherein X_{104} is alanine and X_{114} is glycine.

182. The method according to claim 181 wherein X_{105} is valine.

183. The method according to claim 182 wherein X_{112} is asparagine.

184. The method according to claim 198 wherein the affinity (K_d) of binding to IR is at least about 10^{-5} M.

185. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figure 8.

186. The method according to claim 185 wherein the sequence comprises ACVWPTYWNCG.

187. An amino acid sequence which binds and IR and comprising an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and wherein at least one of X_{104}, X_{105}, X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

172. The amino acid sequence according to claim 167 wherein X_{92} is phenylalanine.

173. The amino acid sequence according to claim 169 wherein the amino acid sequence is HPPLSX_{86} LX_{88} X_{89} X_{90} LL.

174. The amino acid sequence according to claim 167 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLL, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.

175. A method modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and wherein at least one of X_{104}, X_{105}, X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

176. The method according to claim 175 wherein at least two of the amino acids of X_{106} through X_{111} are tryptophan which are separated from each other by at least two amino acids.

177. The method according to claim 176 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.

178. The method according to claim 177 wherein the amino acid sequence comprises WPTYW.

165. The method according to claim 158 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLL, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.

166. An amino acid sequence which binds IR, said amino acid sequence comprising $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88}, X_{89}, X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.

167. The amino acid sequence according to claim 166 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.

168. The amino acid sequence according to claim 167 wherein X_{83} is proline.

169. The amino acid sequence according to claim 167 wherein X_{85} is serine.

170. The amino acid sequence according to claim 167 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.

171. The amino acid sequence according to claim 167 wherein X_{87} is leucine.

157. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88}, X_{89}, X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.

158. The method according to claim 157 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.

159. The method according to claim 158 wherein X_{83} is proline.

160. The method according to claim 158 wherein X_{85} is serine.

161. The method according to claim 158 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.

162. The method according to claim 158 wherein X_{87} is leucine.

163. The method according to claim 158 wherein X_{92} is phenylalanine.

164. The method according to claim 160 wherein the amino acid sequence is $HPPLSX_{86}\ LX_{88}\ X_{89}\ X_{90}\ LL$.

148. The amino acid sequence according to claim 147 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.

149. The amino acid sequence according to claim 148 wherein X_{66} is glutamic acid.

150. The amino acid sequence according to claim 149 wherein X_{63} is leucine.

151. The amino acid sequence according to claim 148 wherein X_{74} is valine.

152. The amino acid sequence according to claim 149 wherein X_{64} is glutamic acid.

153. The amino acid sequence according to claim 148 wherein X_{75} is a tyrosine.

154. The amino acid sequence accord to claim 148 wherein the amino acid sequence comprises WLDQEWAWVQCEVYGRGCPS.

155. The amino acid sequence according to claim 148 wherein the affinity (K_d) of binding to IR is at least 10^{-5} M.

156. The amino acid sequence according to claim 148 wherein the amino acid sequence comprises a sequence selected from the sequences of Figures 6A-6F.

140. The method according to claim 139 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.

141. The method according to claim 140 wherein X_{66} is glutamic acid.

142. The method according to claim 141 wherein X_{63} is leucine.

143. The method according to claim 140 wherein X_{74} is valine.

144. The method according to claim 141 wherein X_{64} is a glutamic acid.

145. The method according to claim 141 wherein X_{75} is a tyrosine.

146. The method accord to claim 140 wherein the amino acid sequence comprises WLDQEWAWVQCEVYGRGCPS.

147. An amino acid sequence which binds IR, said amino acid sequence comprising X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid; X_{63}, X_{70}, and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

134. An amino acid sequence which binds IR, said amino acid sequence comprising X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} $X_{56}X_{57}X_{58}X_{59}$ X_{60} X_{61} wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{60} and X_{61} are any amino acid; X_{43}, X_{46}, X_{49}, X_{50} and X_{54} are hydrophobic amino acids; X_{47} and X_{59} are cysteines; X_{48} is a polar amino acid; and X_{51} ,X_{52} and X_{57} are small amino acids.

135. The amino acid sequence according to claim 134 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} ,X_{52} and X_{57} are glycine.

136. The amino acid sequence according to claim 135 wherein X_{48} is glutamic acid and X_{50} is phenylalanine.

137. The amino acid sequence according to claim 136 wherein the amino acid sequence comprises X_{43} X_{44} X_{45} LCE X_{49} FGG X_{53} X_{54} X_{55} X_{56} G X_{58} C X_{60} X_{61}.

138. The amino acid sequence according to claim 137 wherein an amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE

139. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid; X_{63}, X_{70}, and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

127. The amino acid sequence according to claim 122 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.

128. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} $X_{56}X_{57}X_{58}X_{59}$ X_{60} X_{61} wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60} and X_{61} are any amino acid; X_{43}, X_{46}, X_{49}, X_{50} and X_{54} are hydrophobic amino acids; X_{47} and X_{59} are cysteines; X_{48} is a polar amino acid; X_{51}, X_{52} and X_{57} are small amino acids.

129. The method according to claim 128 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} ,X_{52} and X_{57} are glycine.

130. The method according to claim 129 wherein X_{48} is glutamic acid and X_{50} is a phenylalanine.

131. The method according to claim 130 wherein the amino acid sequence is X_{42} X_{43} X_{44} X_{45}LCE X_{49} FGG X_{53} X_{54} X_{55} X_{56}GX_{58}C X_{60} X_{61}.

132. The method according the claim 131 wherein the amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE.

133. The method according to claim 128 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

120. The method according to claim 116 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.

121. The method according to claim 116 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.

122. An amino acid sequence which binds IR, said amino acid sequence comprising $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22}, X_{25}, X_{26}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{37}, X_{38}, X_{40} and X_{41} are any amino acid, X_{23} is any hydrophobic amino acid,X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid, and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40}.

123. The amino acid sequence according to claim 122 wherein X_{24} and X_{39} are cysteines.

124. The amino acid sequence according to claim 123 wherein X_{23} is selected from methionine, valine, and leucine; X_{27} is selected from glutamic acid, alanine, glycine, glutamine, aspartic acid and valine; X_{31} and X_{32} are small amino acids; and X_{36} is an aromatic amino acid.

125. The amino acid sequence according to claim 122 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

126. The amino acid sequence according to claim 124 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.

114. An amino acid sequence which binds Site 1 of IGF-1R from mammalian cells such that binding to IR is at or below background, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

115. The amino acid sequence according to claim 114 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.

116. A method of binding to Site 2 of IR from mammalian cells, said method comprising contacting said cells with an amino acid sequence comprising $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22}, X_{25}, X_{26}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{37}, X_{38}, X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40}.

117. The method according to claim 116 wherein X_{24} and X_{39} are cysteines.

118. The method according to claim 117 wherein X_{23} is selected from leucine, isoleucine, methionine and valine; X_{27} is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X_{31} is tryptophan, X_{32} is glycine; and X_{36} is any aromatic amino acid.

119. The method according to claim 118 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

106. The amino acid sequence according to claim 102 wherein amino acid X_{18} is tryptophan.

107. The amino acid sequence according to claim 103 wherein X_{20} is tyrosine.

108. The amino acid sequence according to claim 107 wherein X_{21} is phenylalanine.

109. The amino acid sequence according to claim 103 wherein X_{15} is a large amino acid.

110. The amino acid sequence according to claim 101 wherein at least one amino acid is a D-amino acid.

111. The amino acid sequence according to claim 65 wherein at least one amino acid is a D-amino acid.

112. The amino acid sequence according to claim 102 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.

113. The amino acid sequence according to claim 112 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

98. A method of binding to Site 1 of IGF-1R from mammalian cells, said method comprising contacting IGF-1R with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

99. The method according to claim 98 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.

100. The method according to claim 98 wherein the amino acid sequence comprises a sequence selected from the sequences in Figures 3A through 3D.

101. An amino acid sequence which binds Site 1 of IR from mammalian cells, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

102. The amino acid sequence according to claim 101 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of phenylalanine and tyrosine.

103. The amino acid sequence according to claim 102 wherein X_{14} and X_{17} are leucine.

104. The amino acid sequence according to claim 102 wherein X_{14} is leucine.

105. The amino acid sequence according to claim 102 wherein X_{17} is leucine.

88. A method of binding to Site 1 of IR from mammalian cells, said method comprising contacting IR with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14}, X_{17}, and X_{18} are hydrophobic amino acids, X_{15}, X_{16}, and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

89. The method according to claim 88 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.

90. The method according to claim 89 wherein X_{14} and X_{17} are leucine.

91. The method according to claim 89 wherein X_{14} is leucine.

92. The method according to claim 89 wherein X_{17} is leucine.

93. The method according to claim 89 wherein X_{20} is tyrosine.

94. The method according to claim 89 wherein X_{21} is phenylalanine.

95. The method according to claim 90 wherein X_{15} is a large amino acid.

96. The method according to claim 89 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.

97. The method according to claim 96 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

85. The amino acid sequence according to claim 65 wherein the sequence comprises the amino acid sequence $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ wherein X_{115} is selected from the group consisting of trytophan, glycine, aspartic acid, glutamic acid and arginine, X_{116} is selected from the group consisting of aspartic acid, histidine, glycine and asparagine, X_{117} and X_{118} are selected from the group consisting of glycine, aspartic acid, glutamic acid, asparagine, and alanine, X_8 is selected from the group consisting of arginine, glycine, glutamic acid and serine, X_{11} is selected from the group consisting of glutamic acid, asparagine, glutamine and tryptophan, X_{12} is selected from the group consisting of aspartic acid, glutamic acid, glycine, lysine, and glutamine, X_{119} is selected from the group consisting of glutamic acid, glycine, glutamine, aspartic acid and alanine, X_{120} is selected from the group consisting of glutamic acid, aspartic acid, glycine and glutamine, X_{121} is selected from the group consisting of tryptophan, tyrosine, glutamic acid, phenylalanine, histidine and aspartic acid, and X_{122} is selected from the group consisting of glutamic acid, aspartic acid, and glycine.

86. The amino acid sequence according to claim 85 wherein X_{115} is tryptophan, X_{117} is selected from glycine, aspartic acid, glutamic acid and asparagine; X_{118} is selected from glycine, aspartic acid, glutamic acid and alanine; X_{11}, X_{119}, X_{120}, and X_{122} are glutamic acid; X_{12} is aspartic acid, and X_{121} is tryptophan or tyrosine.

87. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8, X_9, X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IR such that binding to IGF-1R is at or below background.

79. The amino acid sequence according to claim 78 wherein X_{100} is an aliphatic amino acid.

80. The amino acid sequence according to claim 79 wherein X_{100} is leucine.

81. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or KDRAFYNGLRDLVGAVYGAWDKK.

82. The sequence according to claim 81 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD.

83. An amino acid sequence comprising an amino acid sequence selected from the group consisting of amino sequences listed in Figures 2A through 2P.

84. An amino acid sequence comprising a sequence selected from the group consisting of

SFYEAIHQLLGV,
NSFYEALRMLSS,
SLNFYDALQLLA,
SSNFYQALMLLS,
SDGFYNAIELLS,
HETFYSMIRSLA,
HDPFYSMMKSLL and
WSDFYSYFQGLD.

70. The amino acid sequence according to claim 68 wherein X_{10} is isoleucine.

71. The amino acid sequence according to claim 68 wherein X_{10} is leucine.

72. The amino acid sequence according to claim 69 wherein X_{13} is leucine.

73. The amino acid sequence according to claim 69 wherein X_9 is tyrosine and X_{10} is phenylalanine.

74. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises an amino acid sequence selected from $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.

75. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises $FYX_8X_9IX_{11}X_{12}L$.

76. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.

77. The amino acid sequence according to claim 74 wherein the amino acid sequence is $FYX_8YFX_{11}X_{12}L$.

78. The amino acid sequence according to claim 65 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.

62. The method according to claim 61 wherein the affinity is between about 10^{-7} M.

63. The method according to claim 48 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or KDRAFYNGLRDLVGAVYGAWD.

64. The method according to claim 48 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 2A through 2P.

65. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8, X_9, X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IGF-1R.

66. The amino acid sequence according to claim 65 wherein the binding occurs at an affinity (K_d) of at least about 10^{-5} M.

67. The amino acid sequence according to claim 66 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.

68. The amino acid sequence according to claim 65 wherein X_6 and X_7 are phenylalanine or tyrosine, and X_{10} and X_{13} are leucine, isoleucine, tryptophan, phenylalanine or methionine.

69. The amino acid sequence according to claim 68 wherein X_6 is phenylalanine and X_7 is tyrosine.

53. The method according to claim 50 wherein X_{13} is leucine.

54. The method according to claim 50 wherein X_9 is tyrosine and X_{10} is phenylalanine.

55. The method according to claim 50 wherein the amino acid sequence is selected from $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.

56. The method according to claim 55 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.

57. The method according to claim 55 wherein the amino acid sequence comprises FYX_8 $YFX_{11}X_{12}L$.

58. The method according to claim 48 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.

59. The method according to claim 58 wherein X_{100} is an aliphatic amino acid.

60. The method according to claim 59 wherein X_{100} is leucine.

61. The method according to claim 48 wherein the amino acid sequence binds to the insulin receptor with an affinity of at least about 10^{-5} M.

$(S295)_2$-14
S-D-G-F-Y-N-A-Acy-E-L-L-S
S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib
G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib
N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib
GRVDWLQRNANFYDWFVAEAcyG-NH_2
and wherein underlined numbers represent a linker as defined in Table 18.

47. An amino acid sequence which specifically binds IR such that binding to IGF-1R is at or below background and wherein said amino acid sequence comprises $X_1X_2X_3X_4X_5$ wherein X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptohpan, tyrosine and phyenylalanine.

48. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence of amino acids $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8, X_9, X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.

49. The method according to claim 48 wherein X_6 and X_7 are selected from group consisting of phenylalanine and tyrosine, and X_{10} and X_{13} are selected from group consisting of leucine, isoleucine, tryptophan, phenylalanine methionine and valine.

50. The method according to claim 48 wherein X_6 is phenylalanine and X_7 is tyrosine.

51. The method according to claim 50 wherein X_{10} is isoleucine.

52. The method according to claim 50 wherein X_{10} is leucine.

14-(GRVDWLQRNANFYDWFVAE LG)
(SDGFYNAIELLSGGG)$_2$-14
H-Acy-CLEE-w-GASL-Tic-QCSG-NH_2
RWPNFYGYFESLLTHFS-NH_2
HYNAFYEYFQVLLAETW-NH_2
EGWDFYSYFSGLLASVT-NH_2
LDRQFYRYFQDLLVGFM-NH_2
WGRSFYRYFETLLAQGI-NH_2
PLCFLQELFGGASLGGYCSG-NH_2
WLEQERAWIWCEIQGSGCRA-NH_2
IQGWEPFYGWFDDVVAQMFEE-NH_2
TGHRLGLDEQFYWWFRDALSG-NH_2
H-Abu-CLEE-w-GASL-Tic-QCSG-NH_2
14-(Dap-CAWPTYWNCG)$_2$
RDHypFYDWFDDi-NH2
S131-14-S209
S294-14-S210
S295-14-S210
S294-14-204
S295-14-S204
GFREGQRWYWFVAQVT-NH_2
VASGHVLHGQFYRWFVDQFALEE-NH_2
VGDFCVSHDCFYGWFLRESMQ-NH_2
DLRVLCELFGGAYVLGYCSE-NH_2
HLSVGEELSWWVALLGQWAR-NH_2
APVSTEELRWGALLFGQWAG-NH_2
ALEEEWAWVQVRSIRSGLPL-NH_2
WLEHEWAQIQCELYGRGCTY-NH_2
AAVHEQFYDWFADQYEE-NH_2
QAPSNFYDWFVREWDEE-NH_2
QSFYDYIEELLGGEWKK-NH_2
DPFYQGLWEWLRESGEE-NH_2
(S204)$_2$-7
(S204)$_2$-9
(S204)$_2$-12
(S204)$_2$-13
DWLQRNANFYDWFVAEL-Lig
Lig-DWLQRNANFYDWFVAEL
(S209)$_2$-9
(S210)$_2$-9
LigKHLCVLEELFWGASLFGYCSGKKKK
KHLCVLEELFWGASLFGYCSGKKKK-Lig
(S294)$_2$-14

SDGFYNAIELLSGGG-Lig
KHLCVLEELFWGASLFGYCSGKK-Lig
AFYDWFAKK-Lig
AFYEWFAKK-NH_2
AFYGWFAKK-NH_2
AFYKWFAKK-NH_2
$(SDGFYNAIELLS\text{-}Lig)_2$-14
$(AFYDWFAKK\text{-}Lig)_2$-14
FHENAYDWFVRQVSKK
FHENFADWFVRQVSKK
FHENFYAWFVRQVSKK
FHENFYDAFVRQVSKK
FHENFTDWAVRQVSKK
FQSLLEELVWGAPLFRYGTG
PLCVLEELFWGASLFGQCSG
QLEEEWAGVQCEVYGRECPS
$Cys\text{-}(Gly)_2$-D117
$(Cys\text{-}(Gly)_2\text{-}D117)_2$
(S210)-14-(S212)
(S131)-14-(S212)
$(S205)_2$-14
$(S204)_2$-14
(S131)-14-(S210)
RVDWLQRNANFYDWFVAELG
VDWLQRNANFYDWFVAELG
DWLQRNANFYDWFVAELG
WLQRNANFYDWFVAELG
LQRNANFYDWFVAELG
QRNANFYDWFVAELG
RNANFYDWFVAELG
NANFYDWFVAELG
ANFYDWFVAELG
NFYDWFVAELG
GRVDWLQRNANFYDWFVAELG-Lig
Lig-GRVDWLQRNANFYDWFVAELG
(S208)-14-(S131)
(S208)-14-(S209)
GRVDWLQRNANFYDWFVAEL
GRVDWLQRNANFYDWFVAE
GRVDWLQRNANFYDWFVA
GRVDWLQRNANFYDWFV
14-$(SDGFYNAIELLS\text{-}Lig)_2$
(GRVDWLQRNANFYDWFVAELG)-14

D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
D117-b-Ala-Orn(b-Ala-D117)
1-(Thia-b-Ala-D117)$_2$
FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
FHENFYDWFVVSK
FHENFYDWFVSK
FHENFYDWFVK
FYDWF-NH_2
FYDWFKK-NH_2
AFYDWFAKK-NH_2
AAAAFYDWFAAAAAKK-NH_2
(D117)$_2$-12
(Cys-Gly-D117)$_2$
Cys-Gly-D117
(D117)$_2$-14
LDALDRLMRYFEERPSL-NH_2
PLAELWAYFEHSEQGRSSAH-NH_2
GRVDWLQRNANFYDWFVAELG-NH_2
NGVERAGTGDNFYDWFVAQLH-NH_2
EHWNTVDPFYFTLFEWLRESG-NH_2
EHWNTVDPFYQYFSELLRESG-NH_2
QSDSGTVHDRFYGWFRDTWAS-NH_2
AFYDWFAK-NH_2
AFYDWFA-NH_2
AFYDWF-NH_2
FYDWDA-NH_2
Ac-FYDWF-NH_2
Lig-FHENFYDWFVRQVSKK
Lig-GGGFHENFYDWFVRQVSKK
FHENFYDWFVRQVSKKGGG-Lig
Lig-CAWPTYWNCG
ACAWPTYWNCG-Lig
ACAWPTYWNCGGGG-Lig
Lig-SDGFYNAIELLS
SDGFYNAIELLS-Lig

44. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVS, DYKDVTFTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.

45. The amino acid sequence according to claim 25 wherein the sequence comprises FHENFYDWFVRQVS.

46. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of

FHENFYDWFVRQVAKK-NH_2
FHENFYDWFVRQASKK-NH_2
FHENFYDWFVRAVSKK-NH_2
FHENFYDWFVAQVSKK-NH_2
FHENFYDWFARQVSKK-NH_2
FHEAFYDWFVRQVSKK-NH_2
FHANFYDWFVRQVSKK-NH_2
FAENFYDWFVRQVSKK-NH_2
AHENFYDWFVRQVSKK-NH_2
fhenfydwfvrqvskk
EFHENFYDWFVRQVSEE
FHENFYGWFVRQVSKK
HETFYSMIRSLAK
SDGFYNAIELLS
SLNFYDALQLLAKK
HDPFYSMMKSLLK
NSFYEALRMLSSK
HPTSKEIYAKLLK
HPSTNQMLMKLFK
HPPLSELKLFLIKK
HAPLSVLVQALLKK
HPSLSDMRWILLK
WSDFYSYFQGLD
D117-Dap(D117)
SSNFYQALMLLS
D117-Dap(CO-CH_2-O-NH_2)
HENFYGWFVRQVSKK
D117-Lys(D117)

36. The amino acid sequence according to claim 25 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence X_{93} X_{94} X_{95} X_{96} X_{97} located at the carboxy terminal end adjacent to X_5 to form $X_1X_2X_3X_4X_5X_{93}X_{94}X_{95}X_{96}X_{97}$, wherein X_{93}, X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.

37. The amino acid sequence according to claim 36 wherein X_{93} is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.

38. The amino acid sequence according to claim 37 wherein X_{96} is leucine or tryptophan.

39. The amino acid sequence according to claim 38 wherein X_{96} is leucine.

40. The amino acid sequence according to claim 39 wherein X_{95} is glutamine, and X_{96} is tryptophan.

41. The amino acid sequence according to claim 40 wherein X_{93} is valine.

42. The amino acid sequence according to claim 41 wherein asparagine is bound to the amino terminal of X_1.

43. An amino acid sequence selected from the amino acid sequences listed in Figures 1-A through 1-O.

27. The amino acid sequence according to claim 25 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.

28. The amino acid sequence according to claim 25 wherein X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptohpan, tyrosine and phyenylalanine.

29. The amino acid sequence according to claim 28 wherein X_3 is selected from the group consisting of aspartic acid and glutamic acid.

30. The amino acid sequence according to claim 29 wherein X_1 and X_5 are phyenylalanine and X_2 is tyrosine.

31. The amino acid sequence according to claim 29 wherein X_4 is tryptophan.

32. The amino acid sequence according to claim 31 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.

33. The amino acid sequence according to claim 31 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.

34. The amino acid sequence according to claim 28 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising $FHENX_1X_2X_3X_4X_5$.

35. The amino acid sequence according to claim 34 wherein the amino acid sequence comprises FHENFYDWF.

DPFYQGLWEWLRESGEE-NH_2
$(S204)_2$-7
$(S204)_2$-9
$(S204)_2$-12
$(S204)_2$-13
DWLQRNANFYDWFVAEL-Lig
Lig-DWLQRNANFYDWFVAEL
$(S209)_2$-9
$(S210)_2$-9
LigKHLCVLEELFWGASLFGYCSGKKKK
KHLCVLEELFWGASLFGYCSGKKKK-Lig
$(S294)_2$-14
$(S295)_2$-14
S-D-G-F-Y-N-A-Acy-E-L-L-S
S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib
G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib
N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib
GRVDWLQRNANFYDWFVAEAcyG-NH_2
and wherein underlined numbers represent a linker as defined in Table 18.

22. The method according to claim 2 wherein the amino acid sequence binds to the insulin receptor with an affinity of at least about 10^{-5} M.

23. The method according to claim 22 wherein the affinity is at least about 10^{-7} M.

24. The method according to claim 23 wherein the affinity is at least about 10^{-9} M.

25. An amino acid sequence comprising $X_1X_2X_3X_4X_5$ wherein X_1, X_2, X_4, and X_5 are aromatic amino acids, X_3 is any polar amino acid, and wherein said amino acid sequence binds to IGF-1R.

26. The amino acid sequence according to claim 25 wherein the IGF-1R binding occurs with an affinity (K_d) of at least about 10^{-5} M.

ANFYDWFVAELG
NFYDWFVAELG
GRVDWLQRNANFYDWFVAELG-Lig
Lig-GRVDWLQRNANFYDWFVAELG
(S208)-14-(S131)
(S208)-14-(S209)
GRVDWLQRNANFYDWFVAEL
GRVDWLQRNANFYDWFVAE
GRVDWLQRNANFYDWFVA
GRVDWLQRNANFYDWFV
14-(SDGFYNAIELLS-Lig)$_2$
(GRVDWLQRNANFYDWFVAELG)-14
14-(GRVDWLQRNANFYDWFVAE LG)
(SDGFYNAIELLSGGG)$_2$-14
H-**Acy**-CLEE-**w**-GASL-**Tic**-QCSG-NH$_2$
RWPNFYGYFESLLTHFS-NH$_2$
HYNAFYEYFQVLLAETW-NH$_2$
EGWDFYSYFSGLLASVT-NH$_2$
LDRQFYRYFQDLLVGFM-NH$_2$
WGRSFYRYFETLLAQGI-NH$_2$
PLCFLQELFGGASLGGYCSG-NH$_2$
WLEQERAWIWCEIQGSGCRA-NH$_2$
IQGWEPFYGWFDDVVAQMFEE-NH$_2$
TGHRLGLDEQFYWWFRDALSG-NH$_2$
H-**Abu**-CLEE-**w**-GASL-**Tic**-QCSG-NH$_2$
14-(Dap-CAWPTYWNCG)$_2$
RDHypFYDWFDDi-NH2
S131-14-S209
S294-14-S210
S295-14-S210
S294-14-204
S295-14-S204
GFREGQRWYWFVAQVT-NH$_2$
VASGHVLHGQFYRWFVDQFALEE-NH$_2$
VGDFCVSHDCFYGWFLRESMQ-NH$_2$
DLRVLCELFGGAYVLGYCSE-NH$_2$
HLSVGEELSWWVALLGQWAR-NH$_2$
APVSTEELRWGALLFGQWAG-NH$_2$
ALEEEWAWVQVRSIRSGLPL-NH$_2$
WLEHEWAQIQCELYGRGCTY-NH$_2$
AAVHEQFYDWFADQYEE-NH$_2$
QAPSNFYDWFVREWDEE-NH$_2$
QSFYDYIEELLGGEWKK-NH$_2$

AFYDWFA-NH_2
AFYDWF-NH_2
FYDWDA-NH_2
Ac-FYDWF-NH_2
Lig-FHENFYDWFVRQVSKK
Lig-GGGFHENFYDWFVRQVSKK
FHENFYDWFVRQVSKKGGG-Lig
Lig-CAWPTYWNCG
ACAWPTYWNCG-Lig
ACAWPTYWNCGGGG-Lig
Lig-SDGFYNAIELLS
SDGFYNAIELLS-Lig
SDGFYNAIELLSGGG-Lig
KHLCVLEELFWGASLFGYCSGKK-Lig
AFYDWFAKK-Lig
AFYEWFAKK-NH_2
AFYGWFAKK-NH_2
AFYKWFAKK-NH_2
$(SDGFYNAIELLS\text{-}Lig)_2$-14
$(AFYDWFAKK\text{-}Lig)_2$-14
FHENAYDWFVRQVSKK
FHENFADWFVRQVSKK
FHENFYAWFVRQVSKK
FHENFYDAFVRQVSKK
FHENFTDWAVRQVSKK
FQSLLEELVWGAPLFRYGTG
PLCVLEELFWGASLFGQCSG
QLEEEWAGVQCEVYGRECPS
Cys-$(Gly)_2$-D117
$(Cys\text{-}(Gly)_2\text{-}D117)_2$
(S210)-14-(S212)
(S131)-14-(S212)
$(S205)_2$-14
$(S204)_2$-14
(S131)-14-(S210)
RVDWLQRNANFYDWFVAELG
VDWLQRNANFYDWFVAELG
DWLQRNANFYDWFVAELG
WLQRNANFYDWFVAELG
LQRNANFYDWFVAELG
QRNANFYDWFVAELG
RNANFYDWFVAELG
NANFYDWFVAELG

NSFYEALRMLSSK
HPTSKEIYAKLLK
HPSTNQMLMKLFK
HPPLSELKLFLIKK
HAPLSVLVQALLKK
HPSLSDMRWILLK
WSDFYSYFQGLD
D117-Dap(D117)
SSNFYQALMLLS
D117-Dap(CO-CH_2-O-NH_2)
HENFYGWFVRQVSKK
D117-Lys(D117)
D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
D117-b-Ala-Orn(b-Ala-D117)
1-(Thia-b-Ala-D117)$_2$
FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
FHENFYDWFVVSK
FHENFYDWFVSK
FHENFYDWFVK
FYDWF-NH_2
FYDWFKK-NH_2
AFYDWFAKK-NH_2
AAAAFYDWFAAAAAKK-NH_2
(D117)$_2$-12
(Cys-Gly-D117)$_2$
Cys-Gly-D117
(D117)$_2$-14
LDALDRLMRYFEERPSL-NH_2
PLAELWAYFEHSEQGRSSAH-NH_2
GRVDWLQRNANFYDWFVAELG-NH_2
NGVERAGTGDNFYDWFVAQLH-NH_2
EHWNTVDPFYFTLFEWLRESG-NH_2
EHWNTVDPFYQYFSELLRESG-NH_2
QSDSGTVHDRFYGWFRDTWAS-NH_2
AFYDWFAK-NH_2

17. The method according to claim 13 wherein X_{95} is glutamic acid and the amino acid sequence is an insulin agonist.

18. The method according to claim 13 wherein asparagine is present as the amino acid bound to the amino terminal of X_1 and wherein $X_1X_2X_3X_4X_5X_{93}$ is FYDWFV

19. The method according to claim 1 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 1, 2, and 9.

20. The method according to claim 1 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVSK, DYKDVTFTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.

21. The method according to claim 1 wherein the sequence is selected from the group consisting of

FHENFYDWFVRQVAKK-NH_2
FHENFYDWFVRQASKK-NH_2
FHENFYDWFVRAVSKK-NH_2
FHENFYDWFVAQVSKK-NH_2
FHENFYDWFARQVSKK-NH_2
FHEAFYDWFVRQVSKK-NH_2
FHANFYDWFVRQVSKK-NH_2
FAENFYDWFVRQVSKK-NH_2
AHENFYDWFVRQVSKK-NH_2
fhenfydwfvrqvskk
EFHENFYDWFVRQVSEE
FHENFYGWFVRQVSKK
HETFYSMIRSLAK
SDGFYNAIELLS
SLNFYDALQLLAKK
HDPFYSMMKSLLK

9. The method according to claim 7 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.

10. The method according to claim 1 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising $FHENX_1X_2X_3X_4X_5$ and possessing insulin agonist activity.

11. The method according to claim 10 wherein the amino acid sequence is FHENFYDWF.

12. The method according to claim 1 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence X_{93} X_{94} X_{95} X_{96} X_{97} located at the carboxy terminal end adjacent to X_5, wherein X_{93}, X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.

13. The method according to claim 12 wherein X_{93} is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.

14. The method according to claim 13 wherein X_{96} is leucine or tryptophan.

15. The method according to claim 14 wherein X_{96} is leucine.

16. The method according to claim 13 wherein X_{95} is glutamine or glutamic acid, and X_{96} is tryptophan.

We claim:

1. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the amino acid sequence $X_1X_2X_3X_4X_5$, wherein X_1, X_2, X_4, and X_5 are aromatic amino acids, and X_3 is any polar amino acid.

2. The method according to claim 1 wherein X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.

3. The method according to claim 2 wherein said amino acid sequence is an insulin agonist.

4. The method according to claim 2 wherein said amino acid sequence is an insulin antagonist.

5. The method according to claim either one of claims 3 or 4 wherein X_1 and X_5 are phenylalanine and X_2 is tyrosine.

6. The method according to claim 5 wherein X_4 is tryptophan.

7. The method according to claim 6 wherein the amino acid sequence is an insulin agonist and X_3 is selected from the group consisting of aspartic acid and glutamic acid.

8. The method according to claim 7 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.

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3. Results

Figure 14 shows the results of one of the experiments. Clearly, IGF-1 and D8 (B12) cause a much slower dissociation rate than the 20E2 (motif 2), H2C (motif 1), C1 (motif 1), and RP6 (motif 2) peptides. This suggests that IGF-1 and D8 (B12) contact IGF-1R in different locations than that of 20E2, H2C, C1, and RP6.

Previous data (EXAMPLE 28) suggests that the motif 6 series binds to a location of IGF-1R that differs from motifs 1 and 2 and that these two sites are not independent of one another. The slowing of the dissociation rate by IGF-1 and D8 (B12) further suggests that there are at least two sites of binding to IGF-1R and that these two sites are not independent of one another.

The following publications, some of which have been cited herein, are cited for general background information and are incorporated by reference in their entirety.

IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocynanin (SA-APC) obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2 [DYKDFYDAIDQLVRGSARAGGTRDKK(ε-biotin)] was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Methods

a. Preparation of the Assay Mix. A 1.25X concentration of Assay Mix consisting of 2.5 nM Eu-labeled IGF-1R, 18.75 nM b-20E2, 2.5 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate.

b. 20 µl of Competitor and Buffer were added to a 96-well microtiter plate (Costar Cat. #3912).

c. Wallac Victor II Fluorometer was readied to read at 665 nm only in multiple repeats (99) of only the wells containing material.

d. 80 µl of the 1.25X Assay Mix was added to the 96-well microtiter plate and promptly placed onto the Victor II for readings.

e. After the original 99 repeat readings were taken, periodic readings were taken until equilibrium had been established.

NOTE: Different conditions can be used for these experiments. For example, a 1.1X concentration of assay mix can be initially made. Then first add 10 µl of Competitor and Buffer to the microtiter plate followed by 90 µl of the Assay Mix.

f. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally competed at 100 µM, whereas IGF-1 was competed at 30 µM. Results are shown in Figure 14.

S173	rB6-4-A12-IR	5620 nM	
RP5	20E2B-3-B3-IR	7450 nM	-
G9	20E2B-1-A6-IGFR	7550 nM	-
RP4	20E2A-4-F9-IR	8110 nM	+
D8 (B12)	D820-4-B12-IR	11300 nM	
RP24	R20b-4-A4-IR	17800 nM	
RP11	A6S Design	18800 nM	+
D8	R20b-4-D8-IR	21650 nM	
A6	R40-3-A6-IGFR	46600 nM	
RP17	R20b-4-A6-IR	50000 nM	
S167	Short A6	~100 μM	
RP3	20E2A-3-B11-IR	~100 μM	-
KC F9	D820-4-F9-IR	~100 μM	
JB3	CONTROL	~100 μM	
KC G1	D820-4-F10-IGFR	~100 μM	
C3-MDM2	CONTROL	>100 μM	
RP21	40F-4-C1-IGFR	>100 μM	
RP22	40F-4-D10-IGFR	>100 μM	
RP23	40F-4-C1-IR	>100 μM	
KC G2	D820-4-F10-IGFR	>100 μM	
KC G7	F815-4-G7-IGFR	>100 μM	

B. IGF-1R Peptide Assay Competition Dissociation

A competition dissociation experiment was performed to determine if any peptides altered the dissociation rate of the 20E2 (B6 motif) peptide in the IGF-1R Peptide Assay. An alteration of the dissociation rate suggests the peptide used in the competition binds to a second site on IGF-1R thus enhancing or slowing the 20E2 dissociation rate through an allosteric interaction.

1. Materials

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of

Compounds begin at 200 μM in a working concentration of 2% DMSO. Controls also contained 2% DMSO.

3. Results

The IC_{50} and holoenzyme phosphorylation activity (see Example 20) values for certain peptides are shown below.

Peptide Data

Name	Sequence	IGF-1R IC_{50}	Holo. Phos.
IGF-1	Natural Ligand	~1-10 nM	
C1	A6S-4-C1-IGFR or D112	~10 nM	
RP9	H2C Design	33 nM	++
20E2	R20a-3-20E2-IR or D118	~100 nM	
G8	20E2B-3-C6-IGFR	139 nM	-
RP2	H2CB-3-B9-IR	163 nM	+
E8	R20b-4-E8-IR or D120	175 nM	
G33	H2CA-4-G9-IGFR	178 nM	+++
RP6	20C-4-G3-IGFR	184 nM	+++++
RP14	H2CA-4-H8-IGFR	225 nM	
S178	B6C-3-C10-IR	240 nM	
RP10	20E2 Design	315 nM	+
S176	A6S-4-G1-IR	418 nM	
H2C	A6S-4-H2-IGFR or D117	~600 nM	+
B6	R40-3-B6-IGFR	631 nM	
RP13	H2CA-4-H6-IGFR	818 nM	
G8	20E2B-3-C6-IGFR	1330 nM	-
S174	R20-4-F9-IGFR	1460 nM	
RP8	20E2 Design	1800 nM	+
S177	B6C-3-C7-IR	2040 nM	
S175	A6S-3-E12-IR	2050 nM	++
RP1	H2CB-4-G11-IR	2790 nM	+
bS175	A6S-3-E12-IR	3230 nM	
NG C2	20E2-3-C2-IGFR	4020 nM	
S179	H2CBa-3-B12-IR	5350 nM	

b. Dilutions of the Competitors were carried out on a 96-well microtiter plate (Costar Cat. #3912). 100 µl of Buffer (TBS pH 8.0 + 0.1 % BSA) were dispensed to wells in columns 1 through 11. Competitors and Buffer were added to Column 12 wells so that the total volume is 150 µl.

c. To identify small organic compounds which also bind the active sites of IGF-1R, dilutions of the small organic compounds are also performed on a 96-well microtiter plate (Costar Cat. #3912). Compounds are dissolved in 100% DMSO. Therefore, 100 µl of Buffer (TBS pH 8.0 + 0.1 % BSA) with 4% DMSO are dispensed to wells in columns 1 through 10. Column 11 contains 100 µl of Buffer with 2.7% DMSO. Compounds (6 µl) are added into 144 µl Buffer (No DMSO) to Column 12 wells.

d. Dilutions were performed across columns on the plate. Once competitors were dispensed into Column 12 and mixed, 50 µl of the solution Column 12 were transferred to wells in 11 and mixed. 50 µl of the Column 11 mixture was transferred to Column 10 wells. This was repeated until 50 µl of Column 3 mixture was transferred to Column 2 wells. Once accomplished to Column 2, 50 µl from Column 2 were removed and discarded. Column 1 wells were reserved for No Competitor Wells. 100 µl volume was therefore maintained across all columns.

e. 50 µl of the Assay Mix was dispensed into wells on a new 96-well microtiter plate. 50 µl from the Dilutions Plate were then added to this plate.

f. 30 µl from Assay Mix Plate were transferred from the 96-well in duplicate on a 384-well microtiter plate (Nunc Cat. # 264512). This covered plate was allowed to incubate at RT overnight.

g. Binding was measured using Wallac's Victor II fluorometer by excitation at 340 nm and measuring emission at 665 and 615 nm.

h. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally diluted starting from 100 µM, where IGF-1 begins at 30 µM.

Exampl 29: IGF-1R Peptide Assays

A. IC_{50} Determinations

Peptides that meet the proper criteria of affinity, selectivity, and activity may be used to develop site-directed assays to identify active molecules which bind to sites on IGF-1R. Assays have been developed using Time-Resolved Fluorescence Resonance Energy Transfer (FRET). These assays are not radioactive, homogeneous (no wash steps), and can be rapidly carried out in 96- or 384-well microtiter plate format facilitating their use in high-throughput screening assays for small organic molecules.

This assay can be used to assess the affinity of peptides for IGF-1R or can be used to find small organic molecule leads in a high-throughput capacity. The determination of the IC_{50} for several peptides is described below.

1. Assay Components

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocynanin (SA-APC) was obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2 [DYKDFYDAIDQLVRGSARAGGTRDKK(ε-biotin)] ("b-20E2") was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Assay Method

a. Preparation of the Assay Mix. A 2X concentration of Assay Mix consisting of 4 nM Eu-labeled IGF-1R, 30 nM b-20E2, 4 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate at RT in the dark for 1-2 h before competitor was added.

times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The values indicate readings taken at OD_{405}. Figure 72A shows competition between cleaved monomers and dimers and F7 phage for binding to Site 2 of IR. Figure 72B shows competition for binding to Site 1 between H2C and the cleaved and uncleaved monomers and dimers. IC_{50} values are shown in Table 20.

TABLE 20

Dimers Site 1/Site 2 IC_{50} Values				
Phage Signal	**H2C**		**F8**	
Cleavage	-	+	-	+
Dimers				
LF-H2C(6)F8	0.2	0.19	0.3	5
LF-H2C(9)F8	0.4	0.11	3	15
LF-H2C(12)F8	0.3	0.19	>16	16
LF-F8 mono	-	-	>20	12
LF-H2C mono	0.145	0.11	>1	>1
H2C mono	0.3	0.2	>0.5	>0.5
MBP-lacZ control	-	-	-	-

- = uncleaved
+ = cleaved

E. Stimulation of autophosphorylation of IR by MBP-Fusion Peptides

Fusion peptides were prepared as described above, and then assayed for IR activation (see Example 20). The results of these experiments shown in Figure 74 indicate that the H2C monomer and H2C-H2C homodimers stimulate autophosphorylation of IR *in vivo*.

H2C dimers (Site 1-Site 1) with a 6 amino acid linker (H2C-6-H2C) were most active in the autophosphorylation assay. Other active dimers are also shown in Figure 74, particularly H2C-9-H2C, H2C-12-H2C, H2C-3-H2C, and F8.

#428 (G3-12-G3)
AQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEW
FESALRKHGAGAAAGAPVPYPDPLEPRAALTN.

#434 (G3-12-G3)
ISEFIEVRAQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRG
GGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.

#437 (H2C)
AQPAMAFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.

#463 (H2C-3-H2C)
AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVPYPD
PLEPRAA.

#435 (H2C-3-H2C-3-H2C)
AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYD
WFVRQVSAAAGAPVPYPDPLEPRAA.

#439 (H2C-6-H2C)
AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAAGAPVP
YPDPLEPRAA.

#436 (H2C-9-H2C)
AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAAG
APVPYPDPLEPRAA.

#449 (H2C-12-H2C)
AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENNFYDWFVRQVS
AAAGAPVPYPDPLEPRAA.

MBP*
ISEFGSSRVDLQASLALAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEA
RTDRPSQQLRSLNGEWQLGCFGG

The MBP- cleaved fusion protein mixtures were appropriately diluted, added to the wells, and incubated at RT for 30 min. An equal volume of F8 or H2C phage displayed peptide was then added to each well and incubated for 1 h. The control wells (100% phage binding) contained only phage and an equal volume of buffer. The control cleaved fusion protein mixture contains a peptide derived from the lacZ gene. The plate was washed 3

Dimers were prepared by expressing them as MBP fusion products. See, Table 1, supra. The sequences of the MBP- cleaved dimers are shown below (core peptide sequences are underlined):

Cleaved Dimer Sequences

#426 (D8)
AQPAMAWLDQEWAWVQCEVYGRGCPSAAAGAPVPYPDPLEPRAA.

#429(D8-6-D8)
AQPAMAWLDQEWAWVQCEVYGRGCPSGGSGGSWLDQEWAWVQCEVY GRGCPSAAAGAPVPYPDPLEPRAA.

#459 (short flag RB6)
ISEFGSADYKDLDALDRLMRYFEERPSLAAAGAPVPYPDPLEPRAA.

#430 (H2C-4-RB6)
DYKDDDDKFHENFYDWFVRQVSGSGSLDALDRLMRYFEERPSLAAAGAP VPYPDPLEPRAA.

#464 (H2C)
DYKDDDDFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.

#446 (F8)
DYKDDDDHLCVLEELFWGASLFGYCSGAAAGAPVPYPDPLEPRAA.

#431 (H2C-6-F8)
DYKDDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSG AAAGAPVPYPDPLEPRAA.

#433 (H2C-9-F8)
DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSHLCVLEELFWGASLFGY CSGAAAGAPVPYPDPLEPRAA.

#432 (H2C-12-F8)
DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGASL FGYCSGAAAGAPVPYPDPLEPRAA.

#452 (G3)
AQPAMARGGGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.

#427 (G3-6-G3)
AQPAMARGGGTFYEWFESTLRKHGAGGGSGGSRGGGTFYEWFESALRK HGAGAAAGAPVPYPDPLEPRAA.
(* A TO T CHANGE)

Dimers were prepared by ligation chemistry (oxime bond in the ligation site) (attached through C-terminal domain).

C. Binding of Dimers to Different IR Constructs Indicates Peptides Bind to Two Independent Sites

Table 19 summarizes the results of binding of phages of D117 (Formula 1 Motif), D123 (Formula 6 motif), D124 (Formula 4 motif), and CP42 (phage expressing peptide J101, Formula 8 motif) monomer to constructs of IR consisting of the L1-cys-L2 region, L1-cys-L2-FnIIIα region and L2-FnIIIα region.

TABLE 19

IR Construct	Peptides Bound	Motif
L1-cys-L2	D117	A6 Only
L1-cys-L2-FnIIIα	D117, CP42, D123, 124	A6, D8, F8
L2-FnIIIα	CP42, D123, D124	D8, F8

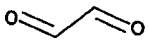
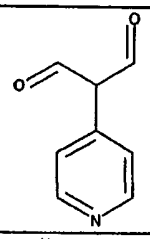
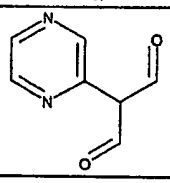
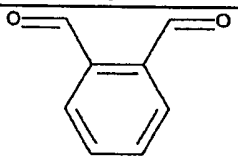
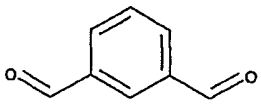
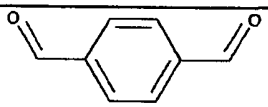
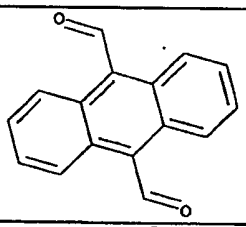
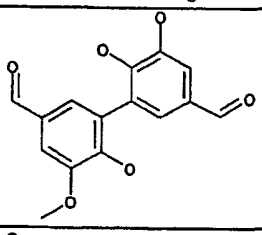
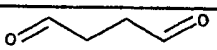
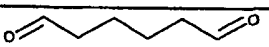
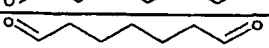
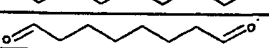
The data above is consistent with a conclusion that the A6 (Formula 1 motif) and F8 (Formula 4 Motif) motifs are physically distinct and on separate parts of IR. Competition data, supra, further indicates that the binding site for the B6 (Formula 2 motif) is on the same subunit as that for the A6 motif.

As shown below, BIAcore competition studies are consistent with the separation of Sites 1 (A6, B6) and 2 (D8, F8, J101).

D. Competition of Site 1and Site 2 Phage Displayed Peptides with Recombinant Cleaved Di-Peptides

Insulin receptor was coated on a 96-well plate with 50 μl of a 2 ng/μl solution of IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h.

TABLE 18

Structure	Number	MW	MW (- $2H_2O$)
	1	100.1	64.1
	2	58.04	22.04
	3	149.15	113.15
	4	150.14	114.14
	5	134.13	98.13
	6	134.13	98.13
	7	134.13	98.13
	8	234.25	198.25
	9	302.3	266.3
	10	72.06	36.06
	11	86.09	50.09
	12	114.14	78.14
	13	128.08	92.08
	14	142.19	106.19

methylmorpholine (37:2:1, v/v/v) under helium. After 2 h. at RT, the resin was washed with 5% in NMP containing 2% diethyldithiocarbamide, Na salt. Finally, the resin was washed with NMP containing HOBt (hydroxybenzotriazole). The protected oxyamino acetic acid (3 mol equiv.) was then coupled on the side-chain of Dap (diaminopropionic acid). The completion of all the acylation reactions was monitored visually by the use of bromophenol blue. Between the Fmoc-deprotection and the acylation reaction, the resin was washed with NMP (x 6).

After synthesis, the peptide was washed with DCM (dichloromethane) (x 3). The peptides were cleaved simultaneously from the resin and the side-chain protecting groups were removed by treatment with 95% aqueous TFA containing triisopropylsilan (TIS) (4 molar equiv.) for 1.5 h. The resin was rinsed with 95% aqueous acetic acid (x 4). Both TFA and acetic acid were evaporated and the peptide was finally precipitated in diethyl ether and lyophilized overnight. The peptide was both analyzed by analytical HPLC and MALDI-MS. Analysis by MALDI-MS; m/z 2287.5 (M + H)+ (requires m/z, 2288.3) confirmed the expected product.

To the peptide monomer, FHENFYDWFVRQVSKK-Dap(CO-CH2-O-NH2) (9.1 mg, 3.9 mol) was added the dialdehyde linker (0.81 mol) dissolved in 80% DMSO (aqueous) (28 l). The pH was then adjusted to 5 with solid sodium acetate. The solution was left overnight at 37°C and progress of the reaction was monitored by RP-HPLC. The formed dimer (see Table 18) was purified by semi-preparative HPLC. Analysis by MALDI-MS confirmed the expected product (see Table 18). The molecular weights and inter peptide distance of various linkers is shown below.

EXAMPLE 28: PREPARATION OF THE DIMERS

A. Materials

Generally, suitably protected N-Fmoc (fluorenylmethoxycarboxyl)-amino acids were purchased from Novabiochem (Switzerland), 1-hydroxy-7-azabenzotriazole (HOAt) from Perspective Biosystems and *N,N'*-diisopropylcarbodiimide (DIC) from Fluka. The molecular weights of the peptides were determined using matrix-assisted laser desorption time of-flight mass spectroscopy (MALDI-MS), recorded on a Voyager-DE (Perseptive Biosystems). A matrix of sinapinic acid was used. Analytical and semi-preparative high-pressure liquid chromatography (HPLC) were performed using a Waters RCM 8 x 10 module and with a C-18 column (19 x 300 mm) and a C-18 column (25 x 300 mm), respectively, at 40°C. The solvent system for both analytical and semi-preparative HPLC was buffer A; 0.1% TFA in water and buffer B; 0.07% TFA in 100% and UV detection was at 215 nm. The gradient for analytical HPLC (1.5 ml/min); a linear gradient of 5-90% buffer B over 25 min and semi-preparative HPLC (4 ml/min); an isocratic gradient of 20% buffer B over 5 min, followed by a linear gradient of 20-60% buffer B over 40 min.

B. Solid-Phase Peptide Synthesis and Analysis of the D117 Monomer(FHENFYDWFVRQVSKK-Dap(CO-CH2-O-NH2)

The peptide monomer available for ligation was synthesized manually in plastic syringes using a preloaded Rink amide linker (RAM)-TentaGel (0.26 mmol/g). Fully protected N-Fmoc amino acids (3 equiv.) were used and the temporary Fmoc protecting group was removed after each cycle by 30% piperidine in *N*-methylpyrrolidone (NMP). The natural amino acids were coupled as their free acids in NMP using DIC (3 mol equiv.) and HOAt (3 mol equiv.) as coupling additive.

First, Fmoc-Dap(Alloc) was coupled as described above. The alloc group was then removed by Pd(0) (3 mol equiv.) in $CHCl_3$/AcOH/N-

TABLE 17

Phage	Sequence Formula Motif	D103 1	D118 2	D119 2	D120 10	D121 10	D122 10	D123 6	D124 4	Insulin
IM332(~J101)	Cyclic	% 85	% 100	% 100	% 100	% 100	% 100	% 100	+ 71	% 98
IM445(~J229)	APTFYAWFNQQT	++ 0	++ 2	++ 0	++ 0	++ 0	% 100	% 100	+ 68	++ 11
IM447(~J227)	SFYEAIHQLLGV	++ 0	++ 0	++ 0	++ 0	++ 0	% 85	+ 58	+ 46	++ 3
IM452(~J228)	HPPLEHLKAFLL	++ 0	++ 0	++ 10	++ 0	++ 0	% 95	nd	% 84	+ 26
IM242(~ILPI)	ILPI	+37	++ 17	+46	+30	+66	+57	+55	++ 19	++ 0

% :>80% signal (not displaced)
+: 20-70% signal
++: <20% signal (fully displaced)

TABLE 16

IM no.	Isolate	Displayed peptide sequence	No. Found		Relative binding to sIR	Relative binding to sIGF-1R-Fc	Formula Motif
IM445	Δ-12-3 #6:	APTFYAWFNQQT-GGGS.	1	[~J229] IC_{50}~2,4 μm	+++	+	1
IM447	Δ-12-3 #45:	SFYEAIHQLLGV-GGGS.	23	[~J227]	+++	+++	2
IM460	Δ-12-3* #76:	NSFYEALRMLSS-GGGS.	2	IC_{50}~6,4 μm	++	(+)	2
IM453	Δ-12-3 #112:	SLNFYDALQLLA-GGGS.	1		++++	++	2
IM466	Δ-12-3* #146	SSNFYQALMLLS-GGGS.	1		+++	++	2
IM448	Δ-12-3 #40	SDGFYNAIELLS-GGGS.	3		+	(+)	2
IM446	Δ-12-3 #24	HETFYSMIRSLA-GGGS.	60		++++	+++	2
IM455	Δ-12-3* #10	HDPFYSMMKSLL-GGGS.	1		+++	++	2
IM465	Δ-12-3 #193	WSDFYSYFQGLD-GGGS.	1		+++	(+)	2
≥50% Consensus:	.	___FY__AI___L__					
IM452	Δ-12-3 #23:	HPPLEHLKAFLL-GGGS.	4	[~J228]	+++++	+	7
IM451	Δ-12-3 #34:	HPPLSELKLFLI-GGGS..	33	IC_{50}~24 μm S124	+++++	+	7
IM459	Δ-12-3* #60:	HPSLSDMRWILL-GGGS.	2		++++	+	7
IM458	Δ-12-3* #30:	HAPLSVLQALL-GGGS.	2		++	+	7
IM449	Δ-12-3 #43:	HPTSKEIYAKLL-GGGS.	14		++	+	7
			4				
IM450	Δ-12-3 #28:	HPSTNQMLMKLF-GGGS.	40		++	+	7
≥50% Consensus:		HPPLS___L__LL		S122 2123			

Δ703 construct and is not displaced from IR by insulin. Accordingly, J101, may bind IR outside of the insulin binding site. J101, which contains two cysteine residues is likely to have a cyclic structure.

Phage displaying IR binding peptides were also identified by binding phage to plates coated with sIR-Fc as discussed above and washing away non-binding phages. Binding phage were eluted with glycine-HCl, pH 2.2 for 10 min.

The sequences of the displayed peptides which bind IR are shown in Figure 8.

A few of the peptides (e.g. J101 and J115) (Figure 8) were tested in the fat cell assay and all were full antagonists.

Exampl 27: Formula 8 synthetic Peptides with Their Affinities for the Human Insulin Receptor (HIR)

A commercial phage display peptide library (New England Biolabs Ph.D.-C7C Disulfide Constrained Peptide Library) was screened for members which bind to IR.

A. Identification of IR Binding Phage

Binding of phage with displayed peptides was detected by ELISA assay. Plates were coated with anti-FC antibody for 2 h at RT or overnight at 4°C. Nonspecific sites were blocked with skim milk (2%) for 1 h at RT. 'sIR-Fc, a modified form of IR in which the cytoplasmic region is substituted with an IgG-Fc fragment (Bass *et al.*, 1990), was then added to the wells for 2 h at RT. Phage were then added to wells and incubated with or without competing peptides for 2 h at RT. Binding was detected with an anti-phage HRP antibody which was added to the wells and incubated for 2.5 h. at RT. OPD (o-phenylenediame) color reaction was detected between 5 and 10 min.

B. Characterization of Phage Displayed Peptides

Fifteen different phage were isolated from a linear 12-mer peptide library (New England Biolabs) panned against a dimer of the LI portion of IR (IR Δ703) (Kristensen *et al.*, 1998) Table 16. The displayed sequences were divided into three groups based on their consensus sequences which correspond to Formula motifs 1, 2 and 7. As can be seen in Table 16, the peptides of motif 7 bind strongly to sIR but not sIGF-1R-FC.

The ability of certain peptides identified in the phage library to compete with other peptides is shown in Table 17 below.

J101 (see Figure 8), the peptide expressed by phage CP42, and containing the Formula 8 motif was found to displace insulin from IR with an IC_{50} of about 5 μm and to be an antagonist in the receptor autophosphorylation and fat cell assays. J101 also does not bind the IR

S266	H-Abu-CLEE-w-GASL-Tic-QCSG-NH_2	$>2*10^{-5}$	0
S267	14-$(Dap-CAWPTYWNCG)_2$		
S268	RDHypFYDWFDDi-NH_2	$4.5*10^{-7}$	0
S273	S131-14-S209	$1.5*10^{-6}$	+
S274	S294-14-S210		
S275	S295-14-S210		
S276	S294-14-204		
S277	S295-14-S204		
S278	GFREGQRWYWFVAQVT-NH_2	$>2*10^{-5}$	0
S279	VASGHVLHGQFYRWFVDQFALEE-NH_2		
S280	VGDFCVSHDCFYGWFLRESMQ-NH_2		
S281	DLRVLCELFGGAYVLGYCSE-NH_2	$1.1*10^{-5}$	0
S282	HLSVGEELSWWVALLGQWAR-NH_2	$>2*10^{-5}$	0
S283	APVSTEELRWGALLFGQWAG-NH_2	$>2*10^{-5}$	0
S284	ALEEEWAWVQVRSIRSGLPL-NH_2	$>2*10^{-5}$	0
S285	WLEHEWAQIQCELYGRGCTY-NH_2	$8.3*10^{-7}$	
S286	AAVHEQFYDWFADQYEE-NH_2		
S287	QAPSNFYDWFVREWDEE-NH_2	$5.9*10^{-6}$	0
S288	QSFYDYIEELLGGEWKK-NH_2	$4.3*10^{-6}$	0
S289	DPFYQGLWEWLRESGEE-NH_2	$>2*10^{-5}$	0
S290	$(S204)_2$-7	$9.0*10^{-7}$	++
S291	$(S204)_2$-9	$1.2*10^{-6}$	++++
S292	$(S204)_2$-12	$7.5*10^{-7}$	++
S293	$(S204)_2$-13	$1.2*10^{-7}$	++
S294	DWLQRNANFYDWFVAEL-Lig	$1.3*10^{-7}$	++
S295	Lig-DWLQRNANFYDWFVAEL	$4.8*10^{-7}$	+
S296	$(S209)_2$-9		
S297	$(S210)_2$-9		
S298	LigKHLCVLEELFWGASLFGYCSGKKKK		
S299	KHLCVLEELFWGASLFGYCSGKKKK-Lig		
S300	$(S294)_2$-14	$5.0*10^{-8}$	+++
S301	$(S295)_2$-14	$6.4*10^{-7}$	+
S302	S-D-G-F-Y-N-A-Acy-E-L-L-S		
S303	S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib		
S304	G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib		
S305	N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib		
S306	GRVDWLQRNANFYDWFVAEAcyG-NH_2		

7, 9, 12, 13, and 14 represent specific chemical linkers (see Table 18)
FFC: 0 is no effect, + is agonist, - is antagonist

S223	FHENFTDWAVRQVSKK	$>2*10^{-5}$	0
S224	FQSLLEELVWGAPLFRYGTG	$>2*10^{-5}$	0
S225	PLCVLEELFWGASLFGQCSG		
S226	QLEEEWAGVQCEVYGRECPS	$1.6*10^{-6}$	
S227	Cys-$(Gly)_2$-D117	$5.1*10^{-7}$	++
S228	(Cys-$(Gly)_2$-D117)$_2$	$3.6*10^{-7}$	++
S229	(S210)-14-(S212)	$4.4*10^{-9}$	0
S230	(S131)-14-(S212)		
S231	$(S205)_2$-14	$2.7*10^{-7}$	+
S232	$(S204)_2$-14	$3.8*10^{-7}$	+++
S233	(S131)-14-(S210)	$2.6*10^{-7}$	+
S234	RVDWLQRNANFYDWFVAELG	$1.3*10^{-7}$	++
S235	VDWLQRNANFYDWFVAELG	$5.3*10^{-8}$	++
S236	DWLQRNANFYDWFVAELG	$1.0*10^{-7}$	++
S237	WLQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S238	LQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S239	QRNANFYDWFVAELG	$1.3*10^{-6}$	0
S240	RNANFYDWFVAELG	$1.4*10^{-6}$	
S241	NANFYDWFVAELG	$1.6*10^{-6}$	
S242	ANFYDWFVAELG	$2.0*10^{-6}$	
S243	NFYDWFVAELG	$2.0*10^{-6}$	
S244	GRVDWLQRNANFYDWFVAELG-Lig	$2.2*10^{-7}$	++
S245	Lig-GRVDWLQRNANFYDWFVAELG	$2.2*10^{-7}$	+
S246	(S208)-14-(S131)	$5.0*10^{-6}$	
S247	(S208)-14-(S209)		
S248	GRVDWLQRNANFYDWFVAEL	$6.3*10^{-8}$	++
S249	GRVDWLQRNANFYDWFVAE	$7.4*10^{-7}$	0
S250	GRVDWLQRNANFYDWFVA	$8.9*10^{-6}$	0
S251	GRVDWLQRNANFYDWFV	$5.6*10^{-6}$	
S252	14-$(SDGFYNAIELLS-Lig)_2$	$4.4*10^{-7}$	0
S253	(GRVDWLQRNANFYDWFVAELG)-14	$2.2*10^{-8}$	++
S254	14-(GRVDWLQRNANFYDWFVAE LG)		
S255	$(SDGFYNAIELLSGGG)_2$-14	$1.6*10^{-6}$	0
S256	H-**Acy**-CLEE-**w**-GASL-**Tic**-QCSG-NH_2	$9.0*10^{-6}$	(-)
S257	RWPNFYGYFESLLTHFS-NH_2	$1.4*10^{-5}$	0
S258	HYNAFYEYFQVLLAETW-NH_2		
S259	EGWDFYSYFSGLLASVT-NH_2	$7.7*10^{-6}$	0
S260	LDRQFYRYFQDLLVGFM-NH_2	$2.3*10^{-6}$	0
S261	WGRSFYRYFETLLAQGI-NH_2	$>2*10^{-5}$	0
S262	PLCFLQELFGGASLGGYCSG-NH_2	$1.9*10^{-5}$	0
S263	WLEQERAWIWCEIQGSGCRA-NH_2	$>2*10^{-5}$	0
S264	IQGWEPFYGWFDDVVAQMFEE-NH_2	$1.9*10^{-7}$	0
S265	TGHRLGLDEQFYWWFRDALSG-NH_2	$1.1*10^{-7}$	0

S160	FHENFYDWFVVSK		
S161	FHENFYDWFVSK		
S162	FHENFYDWFVK		
S165	FYDWF-NH_2	$>2*10^{-5}$	0
S166	FYDWFKK-NH_2	$>2*10^{-5}$	0
S167	AFYDWFAKK-NH_2	$>2*10^{-5}$	(-)
S168	AAAAFYDWFAAAAAKK-NH_2	$3.8*10^{-6}$	0
S169	$(D117)_2$-12	$5.8*10^{-7}$	++
S170	$(Cys\text{-}Gly\text{-}D117)_2$	$7.0*10^{-7}$	+++
S171	Cys-Gly-D117	$2.9*10^{-6}$	+++
S172	$(D117)_2$-14	$4.8*10^{-6}$	+++
S173	LDALDRLMRYFEERPSL-NH_2	$1.2*10^{-6}$	0
S174	PLAELWAYFEHSEQGRSSAH-NH_2	$1.6*10^{-5}$	0
S175	GRVDWLQRNANFYDWFVAELG-NH_2	$2.3*10^{-7}$	+++
S176	NGVERAGTGDNFYDWFVAQLH-NH_2	$4.7*10^{-7}$	+
S177	EHWNTVDPFYFTLFEWLRESG-NH_2	$2.7*10^{-6}$	0
S178	EHWNTVDPFYQYFSELLRESG-NH_2	$1.3*10^{-7}$	++
S179	QSDSGTVHDRFYGWFRDTWAS-NH_2	$5.4*10^{-7}$	+
S180	AFYDWFAK-NH_2	$>2*10^{-5}$	0
S181	AFYDWFA-NH_2	$>2*10^{-5}$	0
S182	AFYDWF-NH_2	$>2*10^{-5}$	0
S183	FYDWDA-NH_2	$>2*10^{-5}$	0
S184	Ac-FYDWF-NH_2	$>2*10^{-5}$	0
S203	Lig-FHENFYDWFVRQVSKK		
S204	Lig-GGGFHENFYDWFVRQVSKK		
S205	FHENFYDWFVRQVSKKGGG-Lig		
S206	Lig-CAWPTYWNCG		
S207	ACAWPTYWNCG-Lig		
S208	ACAWPTYWNCGGGG-Lig		
S209	Lig-SDGFYNAIELLS		
S210	SDGFYNAIELLS-Lig		
S211	SDGFYNAIELLSGGG-Lig		
S212	KHLCVLEELFWGASLFGYCSGKK-Lig		
S213	AFYDWFAKK-Lig		
S214	AFYEWFAKK-NH_2	$>2*10^{-5}$	0
S215	AFYGWFAKK-NH_2	$>2*10^{-5}$	0
S216	AFYKWFAKK-NH_2	$>2*10^{-5}$	0
S217	$(SDGFYNAIELLS\text{-}Lig)_2$-14	$3.9*10^{-8}$	++
S218	$(AFYDWFAKK\text{-}Lig)_2$-14	$1,1*10^{-5}$	0
S219	FHENAYDWFVRQVSKK	$>2*10^{-5}$	0
S220	FHENFADWFVRQVSKK	$>2*10^{-5}$	0
S221	FHENFYAWFVRQVSKK	$1.1*10^{-6}$	(+)
S222	FHENFYDAFVRQVSKK	$>2*10^{-5}$	0

TABLE 15

Name	Sequenc	HIR affinity mol/l	FFC
S105	FHENFYDWFVRQVAKK-NH_2	$3.1*10^{-7}$	++
S106	FHENFYDWFVRQASKK-NH_2	$4.2*10^{-7}$	++
S107	FHENFYDWFVRAVSKK-NH_2	$10.0*10^{-7}$	+
S108	FHENFYDWFVAQVSKK-NH_2	$7.5*10^{-7}$	+
S109	FHENFYDWFARQVSKK-NH_2	$2.3*10^{-7}$	++
S110	FHEAFYDWFVRQVSKK-NH_2	$2.2*10^{-7}$	++
S111	FHANFYDWFVRQVSKK-NH_2	$3.3*10^{-7}$	0
S112	FAENFYDWFVRQVSKK-NH_2	$6.1*10^{-7}$	+
S113	AHENFYDWFVRQVSKK-NH_2	$5.9*10^{-7}$	+
S114	fhenfydwfvrqvskk	$8.3*10^{-6}$	0
S115	EFHENFYDWFVRQVSEE	$6.5*10^{-7}$	+
S116	FHENFYGWFVRQVSKK	$1.4*10^{-6}$	++
S117	HETFYSMIRSLAK	$2.7*10^{-6}$	0
S118	SDGFYNAIELLS	$2.4*10^{-6}$	+
S119	SLNFYDALQLLAKK	$1.8*10^{-6}$	0
S120	HDPFYSMMKSLLK	$2.0*10^{-6}$	0
S121	NSFYEALRMLSSK	$3.1*10^{-6}$	0
S122	HPTSKEIYAKLLK	$9.3*10^{-6}$	0
S123	HPSTNQMLMKLFK	$1.6*10^{-5}$	0
S124	HPPLSELKLFLIKK	$2.3*10^{-5}$	0
S125	HAPLSVLVQALLKK		0
S126	HPSLSDMRWILLK		
S127	WSDFYSYFQGLD	$1.2*10^{-6}$	0
S128	D117-Dap(D117)	$1.1*10^{-6}$	++
S129	SSNFYQALMLLS	$2.9*10^{-6}$	0
S131	D117-Dap(CO-CH_2-O-NH_2)	$1.2*10^{-6}$	+
S137	HENFYGWFVRQVSKK	$7.7*10^{-7}$	0
S145	D117-Lys(D117)	$1.5*10^{-6}$	++
S147	D117-b-Ala-Lys(D117)	$9.3*10^{-7}$	++
S148	D117-b-Ala-Dap(b-Ala-D117)	$1.1*10^{-6}$	++
S149	D117-Gly-Lys(Gly-D117)	$2.0*10^{-6}$	++
S150	D117-b-Ala-Lys(b-Ala-D117)	$6.2*10^{-7}$	++
S152	D117-Dab(D117)	$5.2*10^{-6}$	+
S153	D117-Orn(D117)	$3.9*10^{-6}$	+
S154	D117-Dap(b-Ala-D117)	$3.6*10^{-6}$	+
S155	D117-b-Ala-Orn(b-Ala-D117)	$2.5*10^{-6}$	++
S156	1-(Thia-b-Ala-D117)$_2$		
S157	FHENFYDWFVRQVS		
S158	FHENFYDWFVRQVSK	$8.1*10^{-7}$	+
S159	FHENFYDWFVQVSK		

Results:

The binding assays showed that most of the peptides completely inhibited insulin binding to HIR with IC_{50}-values ranging from 0.3 to 20 μM. One peptide (D124) was active at lower concentration but only displaced insulin partially (see Figure 71). One peptide (D112) had high affinity for HIGF-1R, but all the others showed 2-20 fold selectivity for HIR (see Figure 71).

In the effect assay (FFC), several of the peptides had no effect, some were antagonists, and a few were agonists reaching a response comparable to that of full insulin stimulation. The ED_{50} for the best peptides (D113 and D117) was around 20-30 μM.

Despite a right shifted does response curve relative to insulin, these peptides represent the first non-insulin compounds ever found to elicit a maximal insulin response by binding to the insulin receptor. Such peptides may be useful for development as therapeutics themselves.

The peptides could also be useful as leads for further characterization of molecular requirements for binding to and activation of IR, and/or as tools for identification of the mechanisms involved in the activation.

Analysis of affinity and activity of another group of peptides is shown in Table 15. In addition to presenting data on the single chain or looped peptide, Table 15 also reports data showing high affinity binding of certain dimers.

TABLE 14

Comp. 1	Resp. 2	#expts 3	ED_{50} 4	Comments 5
D101	0	4		
D102	0	2		Precipitates
D103	0	2		
D104	0	2		Precipitates
D105	0	2		
D106	0	2		Precipitates
D107	-2	2		
D108	-1	2		
D110	-1	2		
D110	-2	4		
D111	0	2		
D112	0	5		Precipitates
D113	+2	7	Approx 20 µM	Insoluble, especially after freeze-thaw, resulting in inconsistent results. Some response at basal insulin.
D114	0	2		
D115	0	3		
D116	+2	4	> 20 µM	Slight effect at basal insulin
D117	+2	8	Approx 20 µM	Precipitates. Under assay conditions, soluble at least up to 20µM (no ppt in microscope, low magnification). Some response at basal insulin.
D117K	+2	2	> 20 µM	
D118	+2	5	Approx 20 µM	Biphasic dose response curve (needs repeating)
D119	+1	2		
D120	-1	4		
D121	-1	3		
D122	-1	6		
D123	-1	5		Precipitates
D124	0	5		Precipitates
D125	0	2		
D126	0	2		

[1] Includes series "A" e.g. D101A
[2] Subjective ranking, on a scale of -2 (antagonist) to +2 (agonist)
[3] Includes experiments run at basal and sub maximal insulin concentrations
[4] Estimated, not calculated values.
[5] "Precipitates" indicates precipitate in diluted stock prior to adding to assay. May be soluble under assay conditions

Exampl 26: Determination f Insulin Agonist Activity Based On ^{3}H-Glucose Uptake into Adipocytes

Insulin increases uptake of ^{3}H glucose into adipocytes and its conversion into lipid. Incorporation of ^{3}H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^{3}H products. The effect of compounds on the incorporation of ^{3}H glucose at a sub-maximal insulin dose was determined, and the results expressed as increase relative to full insulin response. The method was adapted from Moody *et al.* (1974).

Mouse epididymal fat pads were dissected out, minced into degradation buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and degraded for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES , 1% HSA) and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED_{20} insulin. The assay was started by addition of ^{3}H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate. Data are presented graphically, as effect of compound on an (approx) ED_{20} insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in figures 71A-71Z; 71A2-71Z2; 71A3-B3. Qualitative references are shown in Table 14.

TABLE 13

Name	K_d (μM) HIR	K_d (μM) HIGF1R	Ratio	Autophosph. Blot
D101	0.51	13	25	-
D102	1.2	7.4	6.2	-
D103	0.74	15	20	-
D104	20	>20		-
D105	2.8	12	4.3	-
D106	0.97	6.2	6.4	-
D107	1.1	9.7	8.8	+
D108	2.3	19	8.3	-
D109	3.6	12	3.3	-
D110	0.84	1.4	1.7	-
D111	0.62	3.2	5.2	-
D112	0.49	0.05	0.1	-
D113	0.75	5.4	7.2	- (prec)
D114	8.1	>20	>2.5	0
D115	8.1	>20	>2.5	0
D116	4.4	8.1	1.8	0
D117	0.70	6.1	8.6	+
D117K	0.82	9.1	11.1	
D118	0.25	1.3	5.2	+
D119	4.5	13	2.9	+
D120	0.37	2.2	5.9	-
D121	1.1	7.4	6.7	-
D122	1.2	>20	>17	0
D123	0.55	16	29	0
D124	0.04*	8.2	200	-
D101A	0.27	11.0	41	
D102A	0.97	16.0	16	
D112A	0.19	0.02*	0.1	
D113A				
D117A	0.60	5.1	8.5	
D119A	3.0	2.5	0.8	
D122A	1.0	>20	>20	
D123A	1.3	>20	>15	
D124A	0.09*	>20	>200	
D125A	2.6	>20	>8	
D126A	1.4	18	13	

TABLE 12

Name	Sequence	Motif
D101	KIGGQGQHQDGNFYDWFVEALAKK	1
D102	KVLQARHGCDSVSDCFYEWFAKK	1
D103	KWSALLSVMDTGFYAWFDDAVKK	1
D104	KGHSWALVRHVDRLFYEWFDLKK	1
D105	KRDKPTDQEEQNWSFYEWFRHKK	1
D106	KVFWNCRSQQLDFYEWFEQAAKK	1
D107	KLESHYVVPQAALDRLFYSWFSKK	1
D108	KFYGWFSRQLSLTPRDDWGLPKK	1
D109	KSAPGLVSNKQDGLFYSWFREKK	1
D110	KRGGGTFYEWFESALRKHGAGKK	1
D111	KDPERMQSDVGFYEWFRAAVGKK	1
D112	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D114	SAKNFYDWFVKK	1
D115	ADKNFYDWFMAAKK	1
D116	DYKDLCQSWGVRIGWLAGLCPKK	9
D117	FHENFYDWFVRQVSKK	1
D117K	KFHENFYDWFVRQVSKK	1
D118	DYKDFYDAIDQLVRGSARAGGTRDKK	2
D119	KDRAFYNGLRDLVGAVYGAWDKK	2
D120	KVRGFQGGTVWPGYEWLRNAAKK	10
D121	KSMFVAGSDRWPGYGVLADWLKK	10
D122	KEIEAEWGRVRCLVYGRCVGGKK	10
D123	KWLDQEWAWVQCEVYGRGCPSKK	6
D124	KHLCVLEELFWGASLFGYCSGKK	4
D101A	KIGGQGQHQDGNFYDWFVEALAKK	1
D102A	KVLQARHGCDSVSDCFYEWFAKK	1
D112A	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113A	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D117A	FHENFYDWFVRQVSKK	1
D119A	KDRAFYNGLRDLVGAVYGAWDKK	2
D122A	KEIEAEWGRVRCLVYGRCVGGKK	10
D123A	KWLDQEWAWVQCEVYGRGCPSKK	6
D124A	KHLCVLEELFWGASLFGYCSGKK	4

for facilitate solubility. Peptides produced recombinantly by phage are indicated as D1XXA.

The peptides are all biotinylated in the side chain of the C-terminal lysine (except D117A). The peptides produced recombinantly are C-terminal acids, whereas the synthetic peptides are C-terminal amides.

The results of the binding and phosphorylation assays are shown in Table 13.

that C1 and H2C at 30 μM inhibit binding to 40-50% of control and C1C at 30 μM inhibit to 60%. B6 and growth hormone do not compete with binding of 12H10 to IR. Four MBP-peptide fusion proteins (D10, 20A4, E7 and H8) all inhibit binding of 12H10 to IR to 20-30 % of control (Figure 69).

Example 24: Effects of Small Organic Molecules on IR Phosphorylation

Organic molecules positive for binding to IGF-1R and negative controls can be tested for their effects on phosphorylation of insulin receptor.

Example 25: Method for Determination of Insulin Receptor Binding of Peptides

In other insulin binding assays, IR was incubated with ^{125}I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after solubilization with Triton X-100. The assay buffer contains 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MSG, 0.5% human serum albumin, 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its ^{125}I-labeled ligand (TyrA14-^{125}I-HI or Tyr31-^{125}I-IGF1) and a dilution series of the substance to be tested was added. After equilibration for 2 days at 4°C, each sample (200 μl) was precipitated by addition of 400 μl 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin and IGF-1 are shown in Figures 71A-71N.

The sequences of certain peptides analyzed are shown in Table 12, except for peptides D125 and D126. Synthetic peptides are numbered D1XX. D117K is an analog of D117 with an extra N-terminal lysine added

(EG&G Wallac) in Tris-buffered saline containing 0.1% BSA is added to 96- or 384-well white low-fluorescence plates (Nunc) for 2 h or overnight. For library screening, 20 μM of small organic molecules in 2 % DMSO are included in the mixture. Unlabeled rVab 43G7 at 50 nM or IGF at 3 μM are used as positive controls. Fluorescence of samples in each well is measured at both 615 nm and 665 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).

Example 22: Binding of Synthetic Peptides to Insulin Receptor

A series of synthetic peptides were synthesized and biotinylated (Anaspec, Inc., San Jose, CA). The binding affinities of these peptides to IR and IGF-1R were tested. Most of these peptides bind to IR at micromolar range (Figure 63). Comparison of binding of biotinylated C1 peptide to IGF-1R and IR is shown in Figure 64, which indicates that binding of C1 to IGF-1R is at the nM range while binding to IR is at the micromolar range. A series of unlabeled peptides or soluble rVab were added to test competition binding to IR (Figure 65). H2C peptide at 30 μM appears to compete for binding to IR with biotinylated peptides from group 1 (Formula 1 motif) (20D1 and 20D3) and the two A6-based peptides (C1 and H2) but not compete with peptides from group 2 (Formula 6 motif) (20A4 and D8), group 3 (Formula 2 motif) (20C11) or the IGF peptide A9. The 33 F7 soluble rVab antibody competes with group 1 and 2 peptides as well as C1 peptide, however, it does not compete with 20C11 or 2A9. Figure 66 shows that H2C competition with biotinylated peptides, 20D3, H2, and C1, binding to IR is dose-dependent. C1C peptide also competes with C1 for IR binding (Figure 67).

Example 23: Competition for Binding to rVab 12H10 by Peptides and MBP-Peptide Fusion Proteins

Several peptides and four MBP-peptide fusion peptides were tested for competition of binding to IR with soluble rVab 12H10. Figure 68 shows

Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 21: Development of IR Assays Using Soluble rVab Antibodies and Biotinylated Peptides

a. Heterogeneous Time-Resolved Fluorescence Assay. Sixty microliters of insulin receptor (60 ng/well) was coated onto 96-well low-fluorescence MaxiSorp (Nunc) plates overnight at 4^0C. The plates were blocked with TBS containing 2% milk and 0.5% BSA for 1 h at RT followed by three TBS washes. To test binding of peptides to insulin receptor, serial dilutions of biotinylated peptides were added to IR coated plates for 2 h to overnight. After TBS wash, europium-labeled streptavidin at 1 μg/ml in assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA; 0.05% bovine Ig; 0.05% NaN_3; 0.01% Tween-20) was added to the plates and incubated for 1 h. To test binding of rVab antibodies to IR, Eu-labeled rVab antibodies in assay buffer were added to the plates and incubated for 2 h to overnight. After incubation with Eu-labeled streptavidin (for peptide test) or europium-labeled rVabs, the plates were washed 5 times with Tris-buffered saline (pH 7.5) containing 0.1% Tween-20 (TTBS) and tapped dry. Sixty microliters of EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).

b. Homogeneous Time-Resolved Fluorescence Assay. A mixture of 27 nM Cy5-labeled rVab 43G7 and 6-8 nM LANCE-labeled IGF-1R

al., 1993; clone 969). The assay procedure begins with the cell stimulation. The IR transfected 32D cells were seeded at 5 x 10^6 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 µM peptide in 50 µl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM Mn_2Cl, 50 µM sodium orthovanadate) containing 10 µM ATP for 45 min at 22°C. The reaction was stopped by adding 50 µl of gel loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc.,

Example 9. Prior to addition of soluble rVabs to the plates, 100 l/well of 100 nM insulin solution in PBS was added to duplicate wells. After incubation for 1 h at RT, the prepared soluble rVabs were added to each well (100 µl/well) without removing the insulin solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described in Example 9.

Example 19: Activities of rVabs in the Cell-Based Assay

Agonistic and antagonistic activities of IR-specific soluble rVabs were tested in 969 cells stably transfected with the gene encoding the human IR and IRS-1 (insulin receptor substrate). The resulting cell line requires IL-3, IL-4, or insulin for growth. Negative control cell lines do not require IRS-1 for growth. The cells were grown in RPMI 1640 media containing 10% FCS and 20 units of IL-3 per ml. Cells were seeded at 30,000 cells/well in 50 µl PRMI1640 (without IL-3) media containing horse serum instead of FCS to reduce the background. Fifty microliters of either insulin or soluble rVabs at different concentrations were added to duplicate wells, followed by incubation for 18 h in a CO_2 incubator. The cell proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt is cleaved to form formazan by the succinate-tetrazolium reductase system that is active only in viable cells. An increase in the number of cells results in an increase of the overall enzymatic activity of the dehydrogenase that results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent were added and the plate was incubated for 1-4 h at 36°C. Figure 60 shows the results of these studies. As can be seen, rVab 12h10 was able to induce an agonist response in 32D cells expressing IR with an ED_{50} of approximately 50 nM.

Example 20: IR Activation Assays

The kinase receptor activation ELISA is a functional assay based on the ability of a sample to stimulate or inhibit autophosphorylation of the insulin receptor construct that has been transfected into 32D cells (Wang *et*

pelleted cells were resuspended in 25 ml of 2xYT-AI media (no glucose is added) and were incubated overnight at 30°C with shaking (250 rpm).

E. Purification of rVabs

The Pharmacia RPSA Purification Module kit was used (Cat. #17-1362-01), and purification was performed according to the manufacturer's directions.

a. A syringe was filled with the Elution Buffer (100 mM glycine, pH 3.0).

b. The stopper on the top of the anti-E-Tag column was removed and a drop of the Elution Buffer was added to the top of the column. The syringe was connected to the column with the Luer adapter. The connection was "drop to drop" to avoid introducing air into the column.

c. The twist-off end was removed and the column was washed with 15 ml of the Elution Buffer at a flow rate of 5 ml/min, followed immediately by 25 ml Binding Buffer (10X Binding Buffer: 0.20 M Phosphate Buffer, 0.05% NaN_3, pH 7.0).

d. Sample was applied with a peristaltic pump P-1 (Pharmacia, Cat. #19-4611-02) at a flow rate of 5 ml/min at 4°C.

e. The column was washed with 25 ml of the Binding Buffer at a flow rate of 5 ml/min to remove unbound *E. coli* proteins.

f. Bound rVab was eluted from the anti-E-Tag column with the Elution Buffer. The first 4.5 ml of material eluted from the column was discarded.

g. The next 5 ml (containing the purified E-tagged rVab) was collected in either one or several fractions.

h. The column was immediately re-equilibrate with 25 ml of the Binding Buffer for use with the next sample.

Example 18: Competition ELISA with rVabs

For IC_{50} determinations, microtiter plates were coated with IR and blocked as in Example 9. Soluble rVabs were prepared as described in

g. Eighty microliters of the MPBS blocking buffer was added to each tube of plate S2 (already containing 320 µl of the supernatant) and incubated for 10 min at RT. This rVab preparation was now ready to be used in an ELISA performed described above.

C. Detection of rVab Binding Using HRP/Anti-E-Tag Conjugate

a. A microtiter plate was coated with the target protein and blocked as previously described. Some of the wells of the microtiter plate were coated with an unrelated antigen to serve as a negative control.

b. The rVab preparation prepared above was diluted two-fold with the MPBS blocking buffer. Two hundred microliters of this solution was added to a set of antigen-coated and control wells.

c. The plate was incubated for 2 h at RT, and then washed 3 times with PBST.

d. The HRP/Anti-E-Tag conjugate was diluted 1:4,000 in the MPBS blocking buffer. Two hundred microliters of the diluted conjugate was added to each well, and the plate was incubated for 1 h at RT.

e. The microtiter plate was washed 3 times with PBST.

f. Two hundred microliters of the ABTS solution was added to each well, the microtiter plate was incubated for 20 min at RT, and the absorbance of each well was read at 405 nm in an appropriate microtiter plate reader.

D. Production of Soluble rVabs

a. A suitable rVab clone in HB2151 cells was transferred from a 2xYT plate to 3 ml of 2xYT-AG media, and the culture was incubated overnight at 30°C with shaking (250 rpm).

b. Part of the overnight culture (2.5 ml) was added to 25 ml of the 2xYT media and incubated for 1 h at 30°C with shaking (250 rpm).

c. The culture was centrifuged at 1000 g for 20 min at RT, and the supernatant was removed from the pelleted cells and discarded. The

b. Fifty microliters of the pool phage from biopanning round 3 (or round 4) were transferred to 2 ml of the log phase HB2151 cells. The cells were incubated with gentle shaking for 1 h at 37°C.

c. The cells were diluted appropriately with the 2xYT media, plated on 2xYT-AGN plates and incubated overnight at 30°C.

B. Preparation of Soluble Antibodies for Screening IGF Repetition

a. Four hundred microliters of 2xYT-AG media were added to each cluster tube (in a rack of 96 tubes in a microtiter format, Costar #4411).

b. The media in cluster tubes were inoculated by transferring the individual well-isolated colonies from the 2xYT-AGN plates using sterile toothpicks; the cluster tubes were then incubated overnight at 30°C with shaking (250 rpm). The array of bacterial cultures in cluster tubes constitutes the Master Plate.

c. The next day, the Master Plate was duplicated by transferring 40 µl of the saturated culture from each tube of the Master Plate to 400 µl of 2xYT-AG medium in a new set of cluster tubes. The new array of duplicated cultures in the microtiter plate format was labeled S1.

d. Plate S1 was incubated for 2 h at 30°C with shaking (250 rpm), and then centrifuged at 1,000 X g for 20 min at RT in a centrifuge equipped with microtiter plate adapters.

e. The supernatant was carefully removed from each cluster tube and discarded to an appropriate waste container. Four hundred microliters of the 2xYT-AI medium (no glucose added) was added to each tube in plate S1, and the plate was incubated overnight at 30°C with shaking (250 rpm).

f. Plate S1 was centrifuged as described above, and 320 µl of each supernatant (containing the soluble recombinant antibodies) was carefully transferred to a corresponding tube in a new set of 96 cluster tubes. The new plate was labeled S2.

by phage preparation according to the Protocol Preparation of Phage described below.

Protocol: Preparation of Phage

The general protocol for phage preparations used to prepare phage displayed rVabs is described below.

1. Phagemid-containing TG1 cells were grown to OD_{600} = 0.5 in 2xYT-AG media at 37°C with shaking (250 rpm).

2. M13K07 helper phage were then added (at MOI = 20), and the cells were incubated for 1 h at 37°C with gentle shaking (150 rpm).

3. Following infection, cells were pelleted by centrifugation at 1,000 g for 20 min and the supernatant containing the helper phage were discarded.

4. The cell pellet was resuspended in the initial culture volume in 2xYT-AK and grown overnight at 30°C with shaking (250 rpm).

5. The cells from the overnight culture were pelleted at 3,000 g for 30 min at 4°C and the supernatant containing the phage was recovered.

6. The supernatant was adjusted to contain 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage was pelleted by centrifugation at 10,000 x g for 30 min. The pellet was resuspended in MPBS.

Example 17: Expression and Characterization of IR Binding rVab Clones

A. Infection of *E. coli* HB2151 Cells

a. To prepare the log-phase cells, 2xYT media was inoculated with *E. coli* strain HB2151 cells (genotype) from a fresh minimal medium plate, and the cells were grown to OD_{600} = 0.5 at 37°C with shaking (250 rpm).

values indicate readings taken at OD_{405} which were normalized as percent control. The control fusion protein MBP-447 contains a peptide that binds the growth hormone receptor. Peptides in bold type contain cysteine residues. Underlined and in bold are values which are $\leq$ 54% of control values.

Example 16: Biopanning the rVab Library

The same rVab library described in Example 5 and panned for members that bound IGF-1R was also panned for members that bind IR. Human insulin receptor was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. The wells were then blocked by adding 100 µl of MPBS to each well and incubating at RT for 1 h.

The phage were incubated with MPBS for 30 min at RT, then 100 µl of the phage solution were added to each well and incubated for 2 h at RT. In the first round, the input phage titer was about 10^{13} cfu/ml. The input phage titer was about 10^{11} cfu/ml in subsequent rounds.

The wells were washed 13 times with 200 µl/well of MPBS, then washed once with PBS (200 µl/well). The bound phage were eluted by adding to each well 100 µl of 20 mM glycine-HCl, pH 2.2. After 30 s, the phage was transferred to an Eppendorf tube and the solution was neutralized by adding 50 µl of 1 M Tris-HCl, pH 8.0, per volume from each well.

TG1 cells were grown to the mid-log phase (OD_{600} = 0.5). Equal volumes of the TG1 cell culture and the neutralized phage solution were mixed together, incubated for 1 h at 37°C without shaking, and then plated onto two 24 cm x 24 cm 2xYT-AG agar plates. The next morning, cells were removed by scraping the surface of the agar plates, and were then suspended in 24 ml 2xYT and stored in 10% glycerol at -80°C.

The input phage for the subsequent rounds of biopanning was prepared by growing 100 µl of the cells from these frozen stocks, followed

were not able to compete to a significant extent any of the phage-displayed peptides from any of the groups. It should be noted that the corresponding identical phage from Group 1 was not tested. The data support the conclusion that the cysteine-containing peptides bind to a contact site (Site 2) which is different than the contact site (Site 1) required for the consensus containing peptides (Group 1, (Formula 1 motif)) to bind the insulin receptor.

TABLE 11

Phage Displayed		Group 1		Group 2		Group 4	Group 7	Control
Peptides		MBP-E7 1.6 µM	MBP-H8 1.6 µM	MBP-A7 (20A4) 5 µM	MBP-D8 2 µM	MBP-D10 4 µM	MBP-F8 2.8 µM	MBP-447 14 µM
Group 1	B8	265	264	329	267	274	240	299
	20 D3	196	196	250	170	218	208	186
Group 2	D8	138	135	53	54	129	111	160
	A7 (20A4)	133	103	28	54	125	21	116
Group 3	20 E2	80	106	100	69	84	161	100
Group 4	A2	92	92	88	74	105	98	79
	D10	92	60	20	20	36	20	117
Group 6	F2	91	97	88	83	92	83	101
	E8	86	75	164	99	94	86	110
Group 7	F8	99	93	44	63	82	43	138
Group 8	40 A2	80	74	118	84	95	80	90

Data reported in the table above was obtained as follows: IR was coated on a 96-well plate with 50 µl of 2 ng/µl IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h. The fusion proteins (mixed #1:5 with MPBS) were added to the wells and incubated at RT for 30 min. An equal volume of phage (displaying various peptides from each of the groups) was then added and incubated for 1.5 h. The control well contained only phage and an equal volume of buffer. The plate was washed 3 times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The

Exampl 15: Insulin Receptor Competition ELISA Using Phage Displayed P ptides and MBP-Peptide Fusion Proteins

To determine whether the binding sites (contact sites) on the insulin receptor for the various peptides are similar, the purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from various groups. Phage-displayed peptides, which were able to bind to IR, were classified into various groups according to consensus sequences identified (see Figures 47 and 48). Peptide sequences of interest were fused to the C-terminus of MBP as previously described. The protein fusion constructs were expressed as soluble proteins, purified, and the protein concentrations were determined. The purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from the various groups as shown in Table 11.

As expected, the fusion proteins containing A7 (20A4), D8, D10, and F8 peptides were able to compete the corresponding identical peptide sequence displayed on phage in the range of 28-54% of the control value. The fusion protein, MBP-A7, was able to significantly compete (<54%) phage-displayed peptides D8, D10, and F8. The other fusion protein from Group 2 (Formula 6 motif), MBP-D8, was able to compete A7 and D10 peptides displayed on phage. Furthermore, the Group 7 (Formula 4 motif) fusion protein MBP-F8 competed A7 and D10 phage displayed peptides. Figures 56A and 56B show the plotted data from Table 11. In Figure 56A, a clear pattern is seen where significant ($\leq$ 54%) competition reactions occur between fusion proteins and phage-displayed peptides which have in common the presence of at least two cysteine residues (see Figures 47 and 48 for peptide sequences).

Also striking is the observation that the cysteine containing fusion proteins were not able to compete phage displayed peptides from Group 1 (Formula 1 motif), which contain the consensus (IGF A6-like) sequences and are without cysteine residues (Figure 56A). In Figure 56B, the fusion proteins containing the Group 1 (Formula 1 motif) consensus sequences

Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block the insulin binding site. The results are shown in Tables 9 and 10. The amino acid sequences referred to in the tables are identified in Figures 47 and 48, except the 447 and 2A9 sequences, which are shown below.

TABLE 9: BIAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 µM)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 µM) + IR	43	Formula 6 Motif
	D8-MBP (1.6 µM) + IR	56	Formula 6 Motif
	D10-MBP (3.4 µM) + IR	81	Formula 11 Motif
	447-MBP (11.5 µM) + IR	195	hGH Pept. Fus.
	MBP (13 µM) + IR	209	Negative Control

TABLE 10: BIAcore Results—Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR +D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

The concentration of each peptide was about 40 µM and the concentration of IR was 450 nM. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA. The 2A9 peptide sequence is: LCQSWGVRIGWLTGLCP.

Glucose	3.00
$(NH_4)_2SO_4$	5.00
$MgSO_4$ •7H2O	0.25
KH_2PO_4	3.00
Citric Acid	3.00
Peptone	10.00
Yeast extract	5.00
pH 6.8	

The culture was grown at 700 rpm, 37°C until the glucose from the medium was consumed (OD_{600} = ~6.0 – 7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50 % glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 μg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain ER2508 (RR1 *lon:mini*Tn*10*(Tet^r) (*malB*) (*argF-lac*)*U169* Pro$^+$ *zjc*::Tn*5*(Kan^r) *fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2 % glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD_{600} of 0.1. When the cultures reached an OD_{600} of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD_{600} of 0.1 and grown at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l):

Example 14: Peptide Fusions to the Maltose Binding Protein - Construction, Purification and Characterization of the Binding to the Insulin Receptor

A. Cloning

The transfer of interesting peptide sequences from phage display to display as maltose binding protein (MBP) -fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 55 shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *Eco*RI and *Hind*III and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG or E-Tag epitopes and also contain the required restriction enzyme sites *Eco*RI and *Hind*III. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick

B. Results

At high insulin dosage, all clones, except F4, G7, and H4 # (not shown), were inhibited; B8 showed the best inhibition, >50%. The apparent lack of binding of F4 (group 1) might be due to the insufficient level of phage present. G7 is a Fc binding phage is should not by inhibited by insulin. H4 is suspected to be a plastic-binding phage. The results are presented in Figure 54.

C. Conclusions

Insulin competition with a representative member from each group indicated that almost all of the groups competed with insulin; only the "plastic binders" and Fc binding phage did not compete. Different degrees of inhibition by these peptides (phage) imply that the peptides recognize different epitopes on or in the close proximity of the receptor active site.

Example 13: Synthetic Peptide 20A4 Competition Results

This experiment was performed as in Example 12. The 20A4 peptide (D122) starting concentration was 58 μM.

Results. The results are included in Table 7. The peptide 20A4 (D122) (A7) competes with Group 2 members (Formula 6 motif), Group 4 member (miscellaneous) D10, and Group 7 member (Formula 4 motif) F8 (D124). There is a partial inhibition of Group 6 member F2. The data is consistent with the conclusion that the site for 20A4 binding is different from the site for Group 1, Group 3, and Group 6.

between the two receptors occurs despite negative results of many of these same phage-displayed peptides.

b. Although the C1 peptide is the most potent inhibitor of phage binding, C1 peptide loses much of its potency advantage over the other peptides binding IR instead of IGF-1R. In addition, A6 gains potency when binding to IR relative to the other peptides. Combined, this suggests that the adjacent surfaces to this active site of the receptors are sufficiently different that peptides and small organic molecules specific for either receptor can be found.

c. The Hill Coefficient of the peptides binding to IGF-1R is always 1.5 to 2-fold higher than the same phage and peptide binding to IR.

Example 12: Competition of Phage Binding with Insulin

Many different peptides isolated from the random peptide libraries were tested for the ability to compete the natural ligand insulin. Clones tested were B8 (D103) (Formula motif 1), F4 (Formula motif 1), A7 (D122) (20A4) (Formula motif 6), D8 (D123; data not shown) (Formula motif 6), C6 (Formula motif 2), E8 (Formula motif 10), H4 (group 5; data not shown), A4 (group 6), G8 (group 7), G7 (Fc binder). H4 most likely binds non-specifically to the material from which the microtiter plate is made.

A. Insulin Competition Procedure

Receptors were coated at 100 µg/ml, 50 µl/well. After blocking with MPBS and washing 3x with PBST, insulin was added in the presence of 0.1% Tween-20 at 2 µM, 100 nM, and 5 nM for 15 min before the addition of IR binding phage. The final concentration of insulin was 1 µM, 50 nM and 2.5 nM. Reaction was incubated at RT for 1 h and wells were washed 3x with PBST (PBS with 0.05% Tween-20). Anti-M13 HRP was added and incubated for 1 h at RT. Wells were washed 3x with PBST before the addition of ABTS. Plates were read at 405 nm.

the serial dilution until Column 2. Fifty microliters were discarded from Column 2 to maintain 100 µl per well. Column 1 was reserved as no peptide wells. The starting working concentrations for each peptide was: H2 – 50 µM; H2C - 100 µM; C1C - 100 µM; D2C – 100 µM; E4 – 33.3 µM; C1 – 50 µM; A6 - 100 µM; and p53 - 100 µM.

B. IGF-1R Peptide Competition

An experiment was designed to ascertain whether IGF-1R peptides have the ability to compete phage that bind to IR. Competition will occur in either IR- or IGF-1R-coated wells. The IGF-1R peptides H2, H2C, C1C, D2C, E4, C1, and A6 were tested for competition with two separate phage. The first, 20D3, (Figures 51A, 51C) is a phage discovered during panning of IR, but is also positive for binding to IGF-1R. The second, H2, (Figures 51B, 51D) is a phage found during panning of the IGF-1R, but is also positive for binding to IR. A p53-like peptide that binds to MDM2 was used as a negative control.

The Hill Plot data are provided in Table 8 below, and presented graphically in Figures 52A-52D.

TABLE 8: Hill Plot Data

	IGF Receptor						Insulin Receptor					
	20D3 Phage			H2 Phage			20D3 Phage			H2 Phage		
Pept.	n	K_d	r^2	n	K_d	r^2	n	K_d	r^2	n	K_d	r^2
H2	1.29	4958	0.991	1.21	9812	0.979	1.07	1133	0.978	0.71	762	0.981
H2C	0.81	5055	0.975	1.02	3720	0.987	1.03	564	0.976	0.62	480	0.926
C1	1.37	19	0.988	0.96	40	0.976	0.83	324	0.999	0.46	132	0.922
C1C	1.32	13475	0.990	1.00	34198	0.945	0.70	1190	0.988	0.53	532	0.956
D2C	1.50	12454	0.995	1.34	33124	0.999	0.81	2491	0.995	0.96	2964	0.983
E4	1.53	6522	0.995	1.11	5868	0.961	0.79	1435	0.979	0.71	387	0.994

C. Observations and Conclusions

a. These peptides can bind to IR and inhibit binding of phage found by either panning IR (20D3) or IGF-1R (H2). This crossover event

Exampl 11: Cross-Reactivity Studies

Phage ELISA experiments show that the IGF-1R peptides H2 and E4 have detectable binding to IR while expressed as a phage fusion. Other IGF-1R-specific peptides such A6, C1, B6, and JBA5 do not have detectable binding to IR when expressed as phage.

A. Experimental Procedures

Phage Production. Each phage culture was started by the addition of 40 µl of the MASTER stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD_{600} ~0.6-1.0. M13K07 helper phage were added to a concentration of ~5 x 10^{10} cfu/ml and incubated at RT for 30 min. The cultures were centrifuged at ~2500g and 4°C for 20 min. The bacterial pellet was resuspended in 20 ml 2xYT-AK and incubated O/N at 37°C. The culture was centrifuged at ~2500 x g and 4°C for 20 min. The supernatant was transferred to new 50 ml centrifuge tubes

Phage ELISA. Each well of the Nunc-Immuno™ plates with the MaxiSorp™ surface were coated with 50 µl of 2 ng/µl either IR or IGF-1R in PBS O/N at 4°C. The wells were blocked with 200 µl of 2% (w/v) Carnation non-fat dry milk in PBS for 1.5 h at RT. Phage were added at 100 µl per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3X with PBST. A solution of 1:3000 diluted HRP:Anti-M13 Conjugate at 100 µl per well of was added for 1 h. Following a repeat of the washing, 100 µl of ABTS was added for 15-30 min. The OD_{405} was measured using a SpectraMax 340 Microplate Spectrophotometer.

Peptide Competition. Peptide Competition Curves were produced during the phage ELISA across rows in triplicate. The stock synthetic peptide solution was added to Column 12 so that the total volume totaled 150 µl (additional phage solution was added when necessary). A serial dilution was made by transferring 50 µl from Column 12 into 100 µl in Column 11, 50 µl from Column 11 into 100 µl in Column 10, and continuing

Observations and Conclusions

1. The C1 and B6 peptides bind to IR. The C1 and B6 peptides expressed as phage-displayed peptides are negatively charged.

2. Groups 1, 3, and 6 (Formulas 1, 2 and 10, respectively), appear to be inhibited by both the C1 and B6 peptides when binding to IR and IGF-1R. All three groups behave with similar characteristics and similar affinities. They all bind to a common site, (Site 1) as shown by competition data.

3. Group 2 (Formula 6 motif) phage clones have different properties despite their sequence similarity. The phage 20A4 is an IR-specific clone. Its binding to IR is not inhibited by C1 or B6 peptides and therefore binds to Site 2. The phage D8 binds to both IR and IGF-1R. Inhibition by C1 peptide and B6 peptide occurs only when binding to IGF-1R. D8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, suggesting an allosteric competition.

4. Some phage appear to have a plastic-binding component (binding to the wells of microtiter plates) in their sequences when high amounts of phage are used. The phage A2, D9-2, H4, 40F10, 40A2, and 40H4 have a significant background to their signals. With the exception of 40H4, all signals increase over this background signal in the presence of IR. The signals for phage A2 and D9-2 also increase over background for IGF-1R. It should be noted the phage for the IGF-1R binder B6 shows this similar characteristic.

5. The Group 2 phage 20A4 and Group 4 phage D10 are specific for IR – there is no detectable binding to IGF-1R. D10 may be inhibited by C1 peptide to a small extent.

6. The phage for Group 7, F8 (Formula 4 motif) has characteristics similar to Group 2, D8 (Formula 6 motif). This clone binds to both IR and IGF-1R, but the C1 and B6 peptides only affect D8 binding when bound to IGF-1R. F8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, (Formula 1, 2 and 10 motifs, respectively).

TABLE 7: Phage Characterization Summary

	Absorbance Values			IR Competitions		sIGF-1R Competitions	
	NFM	sIGF-1R	IR	C1	B6	C1	B6
Group 1							
20D3	0.09	2.26	1.29	Y	Y	-	
B8	0.10	2.55	1.30	Y	Y	-	
Group 2							
20A4	0.15	0.21	1.61	N	N	-	-
D8	0.09	2.19	1.42	N	N	Y	Y
Group 3							
20E2	0.11	2.15	1.01	Y	Y	-	
Group 4							
D10	0.12	0.14	0.73	N*	N	-	-
A2	1.35	2.00	1.79	N	N	N	
Group 5							
D9-2	1.02	2.53	1.64	N	N	-	-
H4	1.16	1.14	1.41	N*	N	-	-
Group 6							
E8	0.10	2.00	1.34	Y	Y	-	
F2	0.09	2.08	1.43	Y	Y	-	
Group 7							
F8	0.14	2.06	1.49	N	N	Y	Y
Group 8							
40A2	0.56	0.55	1.90	Y*	Y	-	-
40H4	0.75	0.83	0.84	-	-	-	-

NFM = Non-fat milk

C1 peptide (D112) has the FYX_3WF Formula 1 motif and an amino acid sequence of DYKDCWARPCGDAANFYDWFVQQASKK

B6 peptide has the $FYX_8X_9LX_{11}X_{12}L$ Formula 2 motif and an amino acid sequence of WNTVDPFYHKLSELLREKK

concentration of the B6 peptide was 68 μM for both receptors. For IR, the starting concentration for the C1 peptide was 48.5 μM. Only 2 μl of the C1 peptide were added to Row A of wells containing IGF-1R. Therefore, the starting concentration was 4.9 μM. The volume was maintained by the addition of 18 μl of the phage solution to Row A.

Natural Ligand Competition. The "Phage First" experiments were performed by adding 10 μl of 5.5 μM, 550 nM, or 55 nM insulin or IGF-1 in PBS to phage-containing wells in the phage ELISA. The working concentrations were 500 nM, 50 nM, and 5 nM. The volume of no ligand wells was maintained by the addition of 10 μl PBS.

The "Ligand First" experiments were performed by added 50 μl of 2 μM, 200 nM, or 20 nM insulin or IGF-1 in PBS containing 0.5% Tween-20 to non-phage containing wells and allowed to incubate 15 min. Fifty microliters of the phage solution was then added to the wells and mixed well. The mixture was allowed to incubate for 2 h at RT and continue with the phage ELISA.

The data are provided in Table 7 and Figures 50A-50D. Sequences were confirmed on all clones by DNA sequencing.

Example 10: ELISA Analyses of Phag

This series of experiments was designed to help characterize the different groups of consensus sequences found during the biopanning of IR. Phage were prepared from each group (two unique sequences each were attempted). Each phage was bound to insulin receptor and competition experiments were performed.

Phage Production. Each phage culture was started by the addition of 30 µl of the master stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD_{600} ~0.6-1.0. M13K07 helper phage were added to a concentration of ~5 x 10^{10} cfu ml^{-1} and incubated at RT for 30 min. The cultures were centrifuged at ~2500g and 4°C for 20 min. The bacterial pellet was resuspended in 30 ml 2xYT-AK. The culture was transferred into 250 ml bottles and incubated O/N at 37°C. The culture was centrifuged at ~2500g and 4°C for 20 min (in 50 ml centrifuge tubes). The supernatant was transferred to new 50 ml centrifuge tubes.

Phage ELISA. Each well of the Nunc-Immuno™ plates with the MaxiSorp™ surface were coated with either 50 µl of 2 ng/µl either IR or sIGF-1R in PBS overnight at 4°C. The wells were blocked with 200 µl of MPBS for 1.5 h at RT. Phage were added at 100 µl per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3 times with PBST. A solution of 1:3000 diluted HRP:Anti-M13 conjugate at 100 µl per well of was added for 1 h. Following a repeat of the washing, 100 µl of ABTS was added for 15-30 min. The OD was measured using a SpectraMax 340 Microplate Spectrophotometer (Molecular Devices) at 405 nm.

Peptide Competition. Competition of phage displayed peptides by the addition of soluble peptides was carried out using the phage ELISA as described above. Twenty microliters of the stock synthetic-peptide solution was added to Row A. A series of 20 µl into 100 µl dilutions were performed until Row G. Twenty microliters were discarded from Row G to maintain 100 µl per well. Row H was reserved as no peptide wells. The starting

formation and glycosylation patterns should mimic the wild-type receptor. The details of this recombinant protein construct are described in Bass *et al.* (1996).

In panning with the peptide library, the IR was immobilized directly onto a protein-binding plastic surface, and four rounds of panning and enrichment were carried out. Analysis of phage clones from rounds three and four showed that 114 of the 216 clones from the 20mer random peptide library and 17 of the 120 from the 40mer random peptide library bound to IR (Figures 1A, 1B, 2A, 2C, 4A, 6A, 10A and 10B). Of those clones tested competitively against insulin for receptor binding, all were blocked by the presence of natural ligand. This result indicated that these phage clones and insulin bind to the same site (or at least overlapping sites) on IR.

Sequence analysis of several clones shows that there are several distinct populations, designated as Groups 1 through 8 (Figures 1-8) (Figures 47 and 48). Several of the peptides based on the sequences for these groups have been chemically synthesized for subsequent testing. Group 1 (Formula 1 motif) peptides contain the consensus sequence FYxWF, and are believed to be agonistic in cell-based assays. Group 2 (Formula 6 motif) is composed of two peptides having a consensus sequence VYGR and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Group 3 (Formula 2 motif) peptides comprise the preferred consensus sequence F-Y-x-A/G-L/I-x-x-L (A/G denotes the alanine or glycine residue, and L/I denotes the leucine or isoleucine residue). Certain Group 3 peptides exhibit agonistic activity in cell-based assays (Figure 49). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin.

Group 7 (Formula 4 motif) is composed of two exemplary peptides which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS containing 0.5% Tween-20 (PBST). Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. An equal volume of log-phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A total of 216 clones from the 20mer library and 120 clones from the 40mer library were picked at random from the third and fourth rounds of panning and screened for IR binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figures 1A, 1B, 2A, 2C, 10A and 10B.

B. One-Day Panning Procedure

Log phase TG1 cells were infected with the eluted phage, amplified in the 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for 1 h at 37°C, the cells were spun down and resuspended in the 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. The overnight phage was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

To isolate specific binders, each library was panned against a soluble form of the human IR. This IR is composed of the extracellular domains of both the α and β chains of the natural receptor, as well as the constant domain from immunoglobulin Fc, retaining the β-α-α-β structure described above. Because the IR is expressed in a eukaryotic system, disulfide bond

TABLE 6

																			M						
															Y				K						
															G				R						
	Characteristic of														T				S						
	IGF-1R Binding														K				D						
	Sequences														s			A		L					
															H			G		A					
															Q			Q		E					
												D		D	Y			E		Q					
	Parental	D	Y	K	D	G	F	R	E	G	N	F	Y	D	W	F	V	A	Q	V	T				
		G				L					T	Q	Q	A	R	L	L		P						
											V	L	F	E	G	V	E								
	Uncharacteristic of										E	V	L	G	P	W	M								
	IGF-1R Binding										G	*	E	R	Q	C	D								
	Sequences										A	Y	S	H	C										
											Q	S	I		S										
											I		N		*										
											Y		K												
											K		*												

Example 9: Panning the Insulin Receptor

A standard method was used to coat and block all microtiter plates. IR (prepared according to Bass *et al.*, 1996) was diluted to 2 µg/ml in PBS. Fifty microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

A. Two-Day Panning Procedure

Eight wells coated with IR were used for each round of panning. One hundred microliters of phage were added to each well. For the first panning round, the input phage titer was 4×10^{13} cfu/ml. For subsequent rounds, the

Example 8:

A composite of amino acid residues observed in sequences of random 20mer, 40mer and A6 (Formula 1) clones is illustrated below:

A6 CORE	N	F	Y	D	W	F
	D6				A	
	E				E9	
	G6				G2	
	H3				Q4	
	K				R	
	P				S	
	Q8					
	S					
	T					
	V					

A summary of preferences for A6 residues is shown in Table 5 below. An illustration of residues which are characteristic of IGF-1R binding sequences (above parental sequence) and those which are not typically associated with binding sequences (below parental sequence). Table 6.

TABLE 5

	X1	X2	X3	X4	X5
1(N)	2(F)	3(Y)	4(D)	5(W)	6(F)
no aromatics; no large aliphatics; no C; no P			no hydrophobics, except tiny; no C; no P		

TABLE 3

				*	*			*			*	
	B6 CORE	D37	P34	**F54**	**Y54**	H12	K15	**L46**	S16	E27	**L53**	L30
5		E	A			A4	A10	I7	A5	A6	M	A6
		G3	D4			D7	G7	M	D3	D3		I
		K4	E			E9	I		E4	G2		K
		R2	G10			G	L8		F5	K		S2
		S5	L			K3	M3		G4	L		T
10		T	Q			L2	N		H	Q3		V13
		V	S			M	Q		L6	R6		
			T			N	R5		M	S4		
						Q7	T		N	V		
						R4	V		Q2			
15						S	W		R2			
						T			T2			
						V			Y2			

Based on the substitutions observed above, the following preferences shown in Table 4 are preferred for substitutions in the amino acid sequence of Formula 2 for binding to IGF-1R.

TABLE 4

25X98	X99	X6	X7	X8	X9	X10	X11	X12	X13	X100
1(D)	2(P)	3(F)	4(Y)	5(H)	6(K)	7(L)	8(S)	9(E)	10(L)	11(L)
no aromatics; no large aliphatics; no c	no aromatics; no c; no + charged			no aromatics; no C; no P; no I	no aromatics, except W; no – charged; no C; no P		no C; no P	no aromatics; no C; no P		no aromatics; no aliphatics; no C; no P

random 20mer library were analyzed in a phage ELISA to identify binders. The DNA of binders was then determined. The results from both libraries show that positions other than positions 3, 4, 7 and 10 as described above can vary relatively at ease (see tables below), while variability at positions 3, 4, 7 and 10 is much more restricted. The results from the B6-2 library show that the restricted core residues were maintained in all binders except one, which happened only in one instance, L (position 7) can be substituted by another hydrophobic residue, M, at that position. The result from the random 20 library panning revealed that another aliphatic amino acid residue, I can substitute for L at position 7. In addition, the restricted residue at position 10 (L) can also be substituted with amino acid residue M. Thus, 2 of the previously identified restricted residues (L at positions 7 and 10) are not absolute, even though L is preferred at these positions. It should be noted that the failure to observe a substitution at a particular residue position does not necessarily indicate that substitutions cannot be made without losing activity, rather such an absence of substitution is indicative of a preference or an aversion for substitution. The findings are summarized below:

B. Results

Combined results from binding clones isolated from B6-2 (doped core) and random 20 libraries of the Formula 2 motif are shown below in Table 3. Sequences from 25 clones from B6-2 and 29 clones from the random 20mer library were analyzed. Numbers adjacent the amino acid residues represent the frequency with which a specific amino acid was observed at the corresponding position.

receptor pairs to be used in a site directed competition binding assay wherein IGF-1R can be used as one member of the pair, with the peptide or a rVab as the other member. Labeling of each member, and detection of pair formation, using either member in radioactive or nonradioactive labeled forms, is possible by a variety of methods known to those skilled in the art of building competition binding assays. This assay provides a high throughput screening assay to identify small organic molecules which bind to the active site of IGF-1R.

Example 7: Phage Library B6-2

This library was designed based on the "core" sequence of the Class I binders Site 1(B6) which posses antagonistic activities in a cell proliferation assay. The core sequence was determined as DPFYHKLSEL, where the residues F (position 3, X_6 of Formula 2), Y (position 4, corresponding to X_7 of Formula 2), L (position 7, corresponding to X_{10} of Formula 2) and L (position 10, corresponding to X_{13} of Formula 2) were the only residues observed at those positions. The purpose of this library was to test the possibility that some binders will show deviations from the core sequence, especially at the positions where substitutions had not previously been observed. The library was therefore made from doped oligonucleotides so that, on average, half of the amino acid residues were altered per peptide. The library was made as described in the original B6 library, i.e., synthetic oligonucleotides were first amplified in a PCR reaction. The resultant products were cloned into pACANTAB5E (Pharmacia) via *Sfi*I and *Not*I restriction sites as previously described. Over 10^{10} different clones were obtained in the final library.

A. Random 20mer Library

1. Panning with the B6-2 and Random 20mer Libraries

The libraries were affinity selected against IGF-1R. 96 clones from round 3 of panning from B6-2 library and 96 clones from round 4 from the

Exampl 6: Agonistic and Antagonistic Activity of IGF-1R-Binding Peptides

Agonistic and antagonistic activities of the IGF-1-specific peptides were tested in FDCP2 cells (NIH) which express IGF-1R. The cell line requires either IL-3 or IGF-1 for growth, and the cells were maintained in RPMI 1640 medium containing 15% FCS (fetal calf serum). Agonism activity assays were performed in a total volume of 100 µl in 96 well plates (flat bottom). Cells were seeded at 30,000 cells/well in 50 µl RPMI 1640 (without IL-3) medium containing 15% FCS in triplicate wells. To each well, 50 µl of a solution containing either IGF-1, rVabs or peptides at different concentrations was added, followed by incubation for 42 h in a CO_2 incubator at 37°C.

Assays to measure the antagonistic activity were performed in a total volume of 100 µl in 96 well plates. An IGF-1-specific peptide, rVab or an appropriate control was added to wells containing 0.003 µM of human IGF-1 and incubated at 37°C for 18 h in CO_2 incubator. Proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt (slightly red) is cleaved to formazan (dark red) by the succinate-tetrazolium reductase system, which is active only in viable cells. An increase in the number of cells results in an increase in the overall activity of the dehydrogenase which results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent was added to each well and the plates incubated for 1-4 h at 37°C. Proliferation was measured by absorbance at 450 nm. Both 5.3 and 5.4 peptides showed an agonistic activity at the 10 µM concentration (Figure 23). Peptides 5.1 and 5.2 showed a significant antagonistic activity in the 3-30 µM concentration range (Figure 22). Control peptide showed no antagonistic activity at the concentrations tested.

The results described demonstrate the feasibility of both the chemical synthesis of and construction of a recombinant expression vector to make sufficient soluble peptide (free or as fusion with some carrier protein) or rVab for testing agonist and antagonist activities. The results provide peptide-

twice background) of 0.05 fmol bIGF-1. The ability of this assay format to detect specifically bound bIGF-1 (or bPeptides) to IGF-1R coated wells was determined.

8. Elisa Analyses

ELISA was performed on selected rVabs. We found that the native IGF-1 ligand inhibits the binding of peptide 5.1 (the sequence of which originates from the phage clone B6) as shown in Figure 43. The detection of the peptide involved a sandwich configuration with the Eu-labeled streptavidin. It was determined that the binding of Eu-labeled rVab 43G7 to IGF-1R is inhibited by IGF-1 with an IC_{50} of approximately 2 nM, as shown in Figure 44. The binding of the biotinylated peptide 5.1 is inhibited by rVab 43G7 with an IC_{50} of about 10 nM (Figure 45), indicating that both the peptide and rVab bind to the same site on the IGF-1R molecule.

Figures 46A-46D demonstrates the binding properties of the 43G7 antibody. The binding of the Eu-labeled 43G7 antibody is competed by peptide 5.1 (clone B6) (Figure 46A) and by the non-labeled 43G7 (Figure 46B), as well as by rVab 39F7 (Figure 46C) and rVab 1G2P (Figure 46D). The sequences of rVabs 1G2P and 39F7 are provided in Figure 35 and Figure 36, respectively.

C. Conclusions

The above results support the use of this assay procedure as a high throughput screen for agents, with affinities for sites on the human IGF-1R which bind IGF-1. The studies show the IGF-1-specific peptides bind in a dose-dependent, saturable manner and are blocked from binding by agents known to bind to the active site of the receptor. This competition is reproducible and easily quantified. Furthermore, the TRFD assay, which is automatable, is much more sensitive than is an ELISA.

labeling efficiency were determined using a Europium standard solution (Wallac).

b. Assay Method

IGF-1R (5 μg/ml in 50 mM $NaHCO_3$) was coated onto low-fluorescence MaxiSorp (Nunc) plates (100 μl/well) overnight at 4°C. The plates were blocked with PBS containing 2% non-fat milk and 0.05% BSA for 2 h at RT, followed by three PBS washes. For competitive ELISA, serial dilutions of unlabelled IGF-1 (0.1 nM-100 μM) were added to the plates (100 μl/well) and incubated at RT for 1-2 h. 100 μl [$Eu3^+$] rVab 43G7 in Wallac's DELFIA assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA, 0.05% bovine Ig; 0.05% NaN_3; 0.01% Tween-20) was added and incubated for 1.5 h at RT. The plates were then washed 5 times with TTBS (TBS buffer containing Tween-20; Wallac) and tapped dry. Subsequently, 100 μl of DELFIA enhancement solution (100 mM acetone-potassium hydrogen phtalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added to each well, and the plates were shaken for 10 min at RT. Fluorescence of each sample well was measured at 615 nm using a DELFIA 1234 fluorometer (EG&G Wallac).

The dose response of TRFD of Eu was studied in microtiter wells. Detection is linear over the range 0.2 to 200 fmol with a limit of detection (twice background) of 0.05 fmol. There are 6010 fluorescent units (FU) per fmol of Eu. Binding and detection of Eu-SA, (4.7 mol Eu/mol streptavidin) to wells coated with biotinylated BSA (bBSA) (6 mol biotin/mol BSA) is linear over the entire range tested. The specific fluorescent activity of streptavidin Eu-SA (with 4.7 mol Eu/mol SA) is 28 kfu/fmol and the limits of detection (i.e., twice background) are 0.030 fmol. Coating with IGF-1R was linear up to inputs of 200 ng/well and thereafter appeared to saturate at about 660 ng bIGF-1 (biotinylated IGF-1) per well. This is the expected amount based on the manufacturer's information about protein saturation densities of these wells (Nunc manual). These studies show a limit of detection of bIGF-1 (i.e.,

7. Time-Resolved Fluorescence Assay

We have selected the basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for IGF-1. In the present assay, occupation of the active site of IGF-1 receptor is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IGF-1 receptor (i.e., IGF-1R:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). We demonstrate the assay using 43G7 rVab or a biotinylated peptide. Furthermore, we show that both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IGF-1R by IGF-1.

In these assays, soluble IGF-1 receptor is coated on the surface of microtiter wells, blocked by PBS containing milk and BSA, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as fre Eu^{+3} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IGF-1R:bP bound saEu is then converted into its highly fluorescent state and detected by TRFD.

a. Preparation of [Eu3+]-Labeled rVab 43G7

One milligram of rVab 43G7 (the sequence is provided in Figure 34) was added to 300 nmol Eu^{3+}-chelated N^1(P-isothiocyanatobenzyl)-diethylenetriamine-N^1,N^2,N^3-tetracetic acid (Wallac). The reaction was conducted at pH 8.5. The tube was mixed gently and placed at ambient temperature. When the reaction was complete (16 h), the sample was diluted 10-fold into the Tris-buffered saline (TBS), pH 7.5, and the separation of the labeled rVab from the unlabeled rVab and free-Eu^{3+} was achieved by using the PD-10 column. The protein concentration and

10,000 g for 15 min. The aqueous phase was discarded, and the pellet resuspended and dialyzed in PBS (phosphate buffered saline, pH 7.4) at 4° C overnight. Insoluble material was removed by centrifugation at 10,000 g, and the supernatant was filtered through a 0.22 μm membrane and purified on an anti-E-Tag antibody affinity column (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with the Elution buffer (100 mM glycine, pH 3.0). The rVab was concentrated to 1 mg/ml, dialyzed against TBS and stored at 4° C. The SDS-PAGE, Western blot analysis and N-terminal sequence analysis of the affinity purified material were performed according to standard protocols.

5. Size Exclusion FPLC Chromatography

The affinity purified rVabs were fractionated by size exclusion FPLC on a Superdex 75 HR10/30 column (Pharmacia) to determine the molecular size and aggregation state of the rVabs. For calibration of the column, High and Low Molecular Weight Gel Filtration Calibration Kits (Pharmacia) were used. Fractions from several chromatographic separations corresponding to a molecular weight of 30 kDa were pooled and concentrated to 0.7-1.0 mg/ml using Amicon XM10 membranes. Protein concentrations were determined using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

6. BIAcore Analyses

IGF-1R was immobilized onto one flow cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocols. BSA was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of the affinity-purified rVabs were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After a subtraction of the background binding (from the control surface), the equilibrium dissociation constant was derived using Scatchard analysis.

Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after a subtraction of appropriate blanks. Phage pools was considered "positive" if the A_{405} of the well was > 2-fold over background.

3. Competition ELISAs

For IC_{50} determinations, microtiter plates were coated with IGF-1R and blocked as described. Phage and soluble rVabs were prepared as described above. Prior to addition of phage or soluble rVabs to the plates, IGF-1 solution in PBS (1 μg/ml) was added to duplicate wells (100 μl/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μl/well) without removing the IGF-1 solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described above.

Six rVab clones bound specifically to IGF-1R. The sequences of the clones are shown in Figure 34-39.

4. Expression And Purification Of Soluble rVabs

E. coli HB2151 carrying the rVab genes on the pCANTAB5E plasmid (Pharmacia) were grown in 2xYT supplemented with 100 μg/ml ampicillin and 1% glucose at 37° C overnight and then subcultured in the absence of glucose at an OD_{600} of 0.1, and grown at 21° C until OD_{600} was 1.0. Expression was induced by the addition of IPTG to 1 mM and the cells were grown for 16 h at 30° C. The cells and culture supernatant were separated by centrifugation and samples of the cell pellet and supernatant were analyzed on a 15% SDS-PAGE gel followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product. The expressed rVabs were purified from the supernatant by precipitation with ammonium sulphate (which was added to 70% saturation) at 21° C, followed by centrifugation at

are agonistic with ED_{50} values of approximately 20 nM (a plot for the 43G7 antibody is shown in Figure 41). Two other rVabs, 1G2P and 39F7, have been shown to be antagonistic, with IC_{50} values of approximately 20 nM (Figure 42).

Microtiter wells were coated with IGF-1R as described above, with eight wells being used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 µl of the phage suspension was added to each well. For the first round, the input phage titer was 8 x 10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 µl /well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 4 cm x 4 cm plates containing 2XYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above.

2. Elisa Analyses Of Phage Pools

To prepare the phage pools, cells from frozen stocks were grown and phage were prepared as described above. Microtiter wells were coated and blocked as described above. The wells were coated with either IGF-1R (R&D Systems, Inc.) or with control BSA. Phage resuspended in MPBS were added to duplicate wells (100 µl/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 µl/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color was developed by addition of ABTS solution (100 µl/well;

uncut DNA. The resulting 800 bp fragment was purified by passing DNA over QIAquick spin columns.

Phagemid pCANTAB 5E (Pharmacia) was digested with the *Sfi*I and *Not*I restriction enzymes, which was followed by the alkaline phosphatase treatment to dephosphorylate the ends of the restriction fragments generated. The digested DNA was purified by running the digested plasmid DNA on a 1% agarose gel, followed by the DNA purification using the QIAEX II (QIAGEN) column. The vector and insert DNA were ligated overnight at 16°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in a electroporation cuvette (0.1 mm gap; 0.5 ml volume, BTX, Inc.). The amount of DNA in one electroporation was 12.5 μg per 500 μl of TG1 electrocompetent cells. Immediately after the pulse, 12.5 ml of a pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added, and the transformants were grown at 37°C for 1 h. The transformants were pooled, the volume measured, and an aliquot was plated onto the 2xYT-G medium containing 100 μg/ml ampicillin (2xYT-AG) plates to determine the total number of transformants. The number of different transformants and the diversity of the library was 3×10^{10}.

The electrocompetent cell preparation, phage library amplification, library phage rescue, phage preparations and coating of microtiter plates were done as described above for the peptide library.

B. Panning for IGF-1R Binders with rVab Antibody Library

1. Panning Procedure

Panning of the antibody library was done essentially as described for the peptide library, for a total of four rounds. Of the 200 clones tested, approximately 10% bound specifically to sIGF-1R. Among these specific binders, 40% can be competed by IGF-1 for receptor binding. The clonal analysis and DNA sequencing (Figures 31-39) followed by ELISA and cell-based assays (Figures 40-46) have shown that two clones, 43G7 and M100,

of the 6-12 amino acids comprising the V_H CDR3 (indicated as "D" in Figure 30).

A total of 49 human genomic v_h genes and ten human genomic v_l genes (Figure 31) were isolated from total human genomic DNA by PCR. The other genetic components of the library (V_h, CDR3, j_h, linker, and j_l) were derived from synthetic oligonucleotides. Assembly of these components was done using directional cloning as outlined in Figure 32 and Figure 33.

A. Ligations

The general schematic for the assembly of the rVab library ("GRABLIB™") is provided in Figure 30. Four gene fragments (VH, VHCDR3/JH/LINKER, VL and JL) were ligated together in the proper orientation and cloned into pCANTAB 5E (Pharmacia). Directional cloning was achieved using the *Bsr*DI restriction enzyme (Figure 32). Forty-nine germline VH segments and ten VL segments encoding many of the genes from the human VH and VL repertoire were isolated (Figure 31) using the polymerase chain reaction. VH CDR3 (ranging from 6 to 12 amino acids) /JH/Linker fragments were generated by ligation of four oligonucleotides (WM 2.1, 2.2, 2.3 and 2.4) and cloning the resulting fragment into the plasmid pUC18 previously cut with *Kpn*I and *Hind*III. The insert was then amplified using PCR and oligonucleotide primers to introduce a synthetic D-segment of 6 to 12 amino acids having a random sequence and the *Bsr*DI restriction site. The JL gene fragments were assembled as a result of annealing of two synthetic oligonucleotides. The assembled fragments (200 ng) were used as template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 800 bp product was purified and concentrated with QIAquick spin columns (QIAGEN), then digested with the *Sfi*I and *Not*I restriction enzymes. Streptavidin-agarose (GibcoBRL) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any

F. TABLE 2: Results of panning with the H5 secondary phage library.

Round	Total	E-Tag$^{+,a}$		IGF-1R$^{+,b}$	
		Number	%	Number	% Total
0	32	22	69 %	0	0 %
1	128	116	91 %	1	1 %
2	128	108	84 %	2	2 %
3	160	116	91 %	65	51 %

[a]E-Tag$^+$ means ELISA absorbance values >2X background. [b]IGF-1R$^+$ means ELISA Absorbance >2X background. Background absorbance values are 0.05 to 0.075.

Each of the IGF-1R$^+$ clones were sequenced, as were 15 IGF-1R$^-$ clones with high E-Tag values (Absorbance >1.0). These sequences are shown in Figure 29. There is no discernible difference between binding sequences and the non-binding sequences with the exception that all of the binding sequences hold the Gly at position 6 constant. All sequences, binding and non-binding, hold the TAG stop codon constant at position 3 (the *E. coli* strain used in phage production contains the *supE44* mutation, therefore Gln replaces the TAG and it denoted in Figure 29 by Q). This suggests TAG stop codon is required for phage production and not binding.

Example 5: Construction of the rVab Recombinant Antibody Variable Region library

The design, expression and purification of single-chain antibodies has been reviewed (Rader and Barbas, 1997; Hoogenboom, 1997). Briefly, the variable portion of the heavy chain (V_H) is linked to the variable portion of the light chain (V_L) by a flexible peptide linker. Random combinations of V_H and V_L genes can be genetically combined to provide some of the diversity required for a library of recombinant variable region antibodies (rVabs) (Figure 30). In our library, further diversity is provided by full randomization

the two libraries (70%) display a peptide (i.e., are positive for E-tag), only about 6% of the clones from the A6 long (A6L) library bind to IGF-1R by phage ELISA, and none of the 24 clones tested from the A6 short (A6S) library bind to IGF-1R. This indicates that the most common outcome of random mutagenesis is the loss of IGF-1R affinity. Nevertheless, some mutants do retain their binding properties and some have improved affinities (see below).

E. Panning with the Secondary Libraries

The two secondary libraries of Example 4 were used in a panning experiment against IGF-1R. Approximately 50 clones from each four rounds of panning were analyzed in a phage ELISA to identify the clones that bind to the receptor. The positive clones were subjected to DNA sequencing and protein sequence comparison. Figure 28 provides a listing of different sequences obtained from panning with the A6S library. The results show that a variety of phage peptide sequences can bind to IGF-1R, while the consensus sequence NFYDWFV is preserved in the majority of instances.

The H5 secondary phage library was panned against IGF-1R to find H5-like peptides with higher affinities for IGF-1R

The H5 Library has a diversity of ~2.6 x 10^{10} clones with a phage titer of 1.0 x 10^{13} phage ml^{-1}. A total of three rounds of panning were performed. Table 2 summarizes the results from the three rounds of panning and shows the ELISA results for the individual clones selected from each round, the number of clones examined in each round of panning, as well as the number and percentage of E-Tag^{+} clones and IGF-$1R^{+}$ clones.

well as versions containing high numbers of mutations per peptide in a very large library (>10^{10}).

Therefore, the H5 secondary mutant library was designed to contain an average of four amino acid changes (mutations) per peptide. The number of possible mutant H5 peptide sequences having four mutations is 1.0×10^{10} and is equivalent to the actual size of the secondary phage library. Sequence analysis indicates that of these peptides 30% have 3-4, 33% have 1-2 and 32 % have 5-6 mutations. There also was a small percent with 7-8 mutations and 5% clones without any mutation.

An oligonucleotide based on the DNA sequence encoding the H5 peptide was synthesized. The sequence of the oligonucleotide is:
5'-CTACAAAGACCTGTGTTAGAGTTTGGGGGTTACGTATCCGGGTTGGT TGGCGGGGGTGGTGTGCGGCGGCCGCAGTGTGA-3'

The underlined base positions were synthesized as mixtures of four nucleosides as follows:

A = 90% A; 3.3% C; 3.3% G; and 3.3% T
C = 3.3% C; 90% C; 3.3% G; and 3.3% T
G = 3.3% C; 3.3% C; 90% G; and 3.3% T
T = 3.3% C; 3.3% C; 3.3% G; and 90% T

Using this oligonucleotide as a template, the H5 secondary library was constructed, electroporated, amplified, and rescued essentially as described for the original peptide library. The final diversity of this secondary library was ~10^{10}.

D. Characterization of Libraries

Forty-eight randomly picked clones from each of the secondary libraries (Round 0, before panning) were rescued and the phage was assayed in an ELISA for binding to the anti-E-tag mAb, as well as for binding to IGF-1R (E-tag is used as an indicator of expression of displayed peptides on phage surfaces). The results showed that although most of the clones in

pCANTAB5E vector as described for the original peptide library. Over 10^{10} different clones were obtained in the final library.

B. Phage Library A6S

While the consensus sequence NFYDWFV was kept constant in the A6S library, the flanking regions were randomized in the A6S library as shown in Figure 26A. The codons in the random region were of the NNK type to reduce the frequency of stop codons (N = A, C, G, or T; K = G or T). The sequence of the synthetic oligonucleotide made is given in Figure 26B. This synthetic oligonucleotide was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfi* I and *Not* I restriction enzymes and cloned into the pCANTAB5E vector as described for the original peptide library. Over 10^9 different clones were obtained in the final library.

C. Secondary Phage Library Based on Clone H5

Peptide H5 (LCQRLGVGWPGWLSGWCA) was identified in an independent experiment as a binder to the rat growth hormone binding protein. This peptide and four other H5-like peptides, including 2C3-60 (Figure 27), were found in cell culture experiments to possess agonistic activity toward IGF-1R$^+$ cells, but not against IGF-1R$^-$ cells. Further, subsequent *in vitro* experiments showed that the H5-like peptides are not competed by IGF. This suggests that these peptides recognize a second allosteric site on IGF-1R. BIAcore analysis showed that binding of the 2C3-60 peptide to IGF-1R is ~20 μM. Subsequently, a phage library of mutants of the H5 sequence was constructed and used for panning against IGF-1R.

Gene synthesis to introduce mutations and phage display were used to construct an H5 secondary library. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes which are phage displayed. This method allowed the encoding of both the original H5 peptide as control as

Exampl 4: Construction of Secondary Phage Librari s

Two phage libraries were designed on the basis of the sequences of the Class II binders known to possess agonistic properties in cell-based assays. The goal was to bring the affinity into a range that would allow the peptide to be used in a receptor binding assay and tested in a cell based assay for activity. Among several available mutagenesis methods, we chose one based on gene synthesis and phage display. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes, the expression products of which are phage displayed.

A. Phage Library A6L

The approach used was the doped synthesis of the oligonucleotide encoding the sequence of the peptide. The sequence encoding the peptide and the sequence of the synthetic oligonucleotide made are shown in Figures 25A-25B. The amino acid residues belonging to the consensus sequence were kept constant and were not mutated. The ratio of nucleosides in each condensation was chosen to provide an average of 6 nucleotide sequence changes at the DNA level and 4-5 mutations at the amino acid level over the length of the peptides. The regions corresponding to the FLAG, *Sfi*I and *Not*I sites were not mutated.

The DNA sequence encoding the A6 peptide was optimized for *E. coli* codon usage by replacing a total of 24 nucleotides as shown in Figure 25A. The TAG stop codons (suppressed in the TG1 *E. coli* strain used) were replaced with CAG (glutamine). Then, the oligonucleotide sequence was designed to include doped nucleosides at positions corresponding to the coding region for the A6 peptide, except for the consensus NFYDWFV (Figure 25A). This synthetic oligonucleotide (Figure 25B) was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfi*I and *Not*I restriction enzymes and cloned into the

F-Y-x-x-L-s-x-L, and are shown to be antagonistic in cell-based assays (Figure 22). Class II peptides contain the consensus N-F-Y-D-W-F-V, and are shown to be agonistic in cell-based assays (Figure 23). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature IGF-1.

Example 3: Assays with Synthetic Peptides

Four synthetic peptides, 5.1, 5.2, 5.3 and 5.4 (Figure 21) were made to study the properties of the artificial peptide ligands from phage display.

Synthetic peptides were obtained from a commercial supplier (Anaspec). The peptides were supplied greater than 90% pure by HPLC. The molecular weights of the peptides as determined by mass spectroscopy agreed with the expected values.

IGF-1R (100 μg/ml) was immobilized onto one flow-cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocol. An unrelated IgG was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of synthetic peptide were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After subtraction of background binding from the control surface, the results were used to derive an equilibrium dissociation constant using Scatchard analysis (Figure 24A).

In another experiment, IGF-1R (100 μg/ml) was immobilized onto a CM-5 sensor chip as described above, and an unrelated IgG was immobilized in the same manner to another flow cell of the same chip. IGF-1 alone, peptide 5.1 alone (corresponding to the B6 phage clone), or different mixes of the two, were injected over the derivatized chip surfaces. The results shown in Figure 24B indicate that the 5.1 peptide inhibits the binding of IGF-1, and the inhibition is increased by increasing amounts of the peptide. The results support the idea of an overlap of the peptide 5.1 binding site and the IGF-1 binding site on IGF-1R.

was developed by addition of ABTS solution (100 µl/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices Corp., Sunnyvale CA) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was $\geq$ 2-fold over background.

For IC_{50} determinations in a competitive ELISA, microtiter plates were coated with IGF-1R and blocked as described. Phage were prepared as described. Prior to addition of phage to plates, the peptide or recombinant variable antibody or fragment ("rVab"), or an appropriate control, was diluted in PBS and added to duplicate wells (100 µl/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 µl/well) without removing the peptide or rVab solution. After incubation for 1 h at RT, the wells were washed and the color developed as described above.

The clones were next analyzed for binding to the receptor's active site (Figures 20A and 20B). Competitions of phage binding were done with the cognate ligand (i.e., IGF-1). All four phage clones tested, B6, F6, C6 and E5, bound to same site as IGF-1 since the binding of the clones to the immobilized IGF-1R could be inhibited with IGF-1.

To determine the rank order for phage peptides, the human IGF-1R (25 g/ml) was immobilized onto a CM-5 (BIAcore) sensor chip using amino coupling chemistry and the manufacturer's recommended protocol. The final surface density was 1000 RU. A monoclonal antibody was immobilized onto another flow cell as a control surface. Phage were directly injected (30-100 µl) with a buffer flow rate of 1 µl/min. Background binding to the control surface was subtracted prior to further analysis.

C. Phage Sequence Analysis

Sequence analysis of several clones shows that there are two distinct populations, designated as Class I (Formula motif 2) and Class II (Formula motif 1; Figure 21). Several of these have been chemically synthesized for subsequent testing. Class I peptides contain the consensus sequence D-x-

Eight wells were used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 µl was added to each well. For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 µl/well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones were picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figure 18. Some of the clones (Figure 19) were frameshifted, that is, the relevant peptide sequence was encoded not in the FLAG frame, but in either frame + 1 or – 1.

B. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps are the same for pooled and clonal phage. Microtiter wells were coated and blocked as described above. Wells were coated with either IGF-1R or a control IgG mAb. Phage resuspended in MPBS were added to duplicate wells (100 µl/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 µl/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color

OD_{600} = 0.5 in 2xYT-AG at 30°C with shaking (250 rpm). Helper phage (M13K07) was then added (multiplicity of infection (MOI) = 15), and the cells were incubated for 30 min at 37° C without shaking, followed by 30 min at 37°C with shaking (250 rpm). Following infection, cells were pelleted and the supernatant containing the helper phage was discarded. The cell pellet was resuspended in the initial culture volume of 2xYT-A (no glucose) containing 50 mg/ml kanamycin and grown overnight at 30°C with shaking (250 rpm). The cells from the overnight culture were pelleted at 3000 x g for 30 min at 4°C and the supernatant containing the phage was recovered. The solution was adjusted to 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage were pelleted by centrifugation at 10,000 x g for 30 min, then resuspended in phosphate-buffered saline (1/100 of the initial culture volume) and passed through a 0.45 μm filter. The phage were titered by infecting TG1 cells. The phage titer for the 40mer peptide library was 4×10^{13} cfu/ml. The phage titer for the 20mer library was 3×10^{-3}.

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the OD_{600} increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

Example 2:

A. Panning IGF-1R

A standard method was used to coat and block all microtiter plates. The soluble IGF-1R ("sIGF-1R") was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h.

et al., 1988) was incorporated into the library as an immunoaffinity tag as shown in Figure 16.

Another phage library expressing 20mer peptides, was constructed according to a similar procedure. The diversity of the library is 1.1×10^{11} different clones.

B. Preparation of Electrocompetent Cells

To prepare electrocompetent cells, an overnight culture of *E. coli* TG1 cells (F'_*traD*36 *lacI*^{q} Δ(*lacZ*)M15 *proAB) / supE* Δ(*hsdM-mcrB*)$_5$ $r_k^- m_k^-$ *McrB*$^-$) *thi* Δ(*lac-proAB*) was diluted to an OD_{600} = 0.05-0.1 in 500 ml 2xYT, then grown at 37°C in 4 liter Ehrlenmyer flasks to an OD_{600} = 0.5-0.6. The culture was poured into pre-chilled centrifuge bottles and incubated on ice for 30 min prior to centrifugation at 2000 x g for 30 min (2°C). The supernatant was poured off and the cell pellet was resuspended in a total of 400 ml of ice cold sterile distilled water. The process of centrifugation and resuspension was repeated 2 times. After the last centrifugation, the pellet was resuspended in a total of 25 ml of ice cold water containing 10% glycerol. The cell suspension was transferred to pre-chilled 35 ml centrifuge bottles, and was then pelleted at 2000 x g for 10 min at 4°C. The cells were then suspended in 0.3 ml of the same 10% glycerol solution, aliquotted into smaller tubes, and snap-frozen on dry ice. The aliquots were stored at -80°C.

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the A_{600} increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

C. Phage Rescue

This process was carried out using the standard phage preparation protocol with the following changes. Five individual recombinant cell libraries, with a total diversity of 1.6×10^{10}, were combined and grown to

Example 1

A. Construction of Phage Library for Identifying IGF-1R and IR Binding Ligands

The schematic for the peptide library "RAPIDLIB™" on filamentous phage is shown in Figure 16. DNA fragments coding for peptides containing 40 random amino acids were generated in the following manner. A 145 base oligonucleotide was synthesized to contain the sequence $(NNK)_{40}$, where N = A, C, T, or G, and K = G or T. This oligonucleotide was used as the template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 190 bp product was purified and concentrated with QIAquick spin columns (QIAGEN, Inc. Valencia, CA), then digested with *Sfi*I and *Not*I. Streptavidin-agarose (GibcoBRL Life Technologies, Inc., Rockville, MD) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any uncut DNA. The resulting 150 bp fragment was again purified over QIAquick spin columns. The phagemid pCANTAB5E (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) was digested with *Sfi*I and *Not*I, followed by phosphatase treatment. The digested DNA was purified using a 1% agarose gel followed by QIAEX II (QIAGEN). The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN). Electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 12.5 μg of DNA and 500 μl of TG1 electrocompetent cells (see below). Immediately after the pulse, 12.5 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were pooled, the volume measured, and an aliquot was plated onto 2xYT-G containing 100 g/ml ampicillin (2xYT-AG) plates to determine the total number of transformants.

Sequence analysis of randomly selected clones indicated that 54% of all clones are in-frame (Mandecki *et al.*, 1997). The FLAG sequence (Hopp

cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

The following non-limiting examples illustrate various aspects and embodiments of the invention and should not be contrived as limiting the scope of the invention.

VI. EXAMPLES

The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996. The insulin is either from Sigma (Cat. # I-0259) or Boehringer. The IGF-1 is from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates are from Nunc via Fisher (Cat. # 12565347). The HRP/Anti-M13 Conjugate is from Pharmacia (Cat. # 27-9421-01). The ABTS solution is from BioF/X (Cat. # ABTS-0100-04).

plasmid) to a protein ligand via polylysine. The appropriate or suitable ligands are selected on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell or tissue type. These ligand-DNA conjugates can be injected directly into the blood, if desired, and are directed to the target tissue where receptor binding and DNA-protein complex internalization occur. Co-infection with adenovirus to disrupt endosome function can be used to overcome the problem of intracellular destruction of DNA.

An approach that combines biological and physical gene transfer methods utilizes plasmid DNA of any size combined with a polylysine-conjugated antibody specifically reactive with the adenovirus hexon protein. The resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector allows efficient binding to the cell, internalization, and degradation of the endosome before the coupled DNA can be damaged.

Many types of cells and cell lines (e.g. primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g. myelocytes, macrophages, monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g. K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary

unit containing the gene to be introduced into a specific cell for replacement gene therapy; a selectable gene as described below; and a 3′ LTR which contains a deletion in the viral enhancer region, or deletions in both the viral enhancer and promoter regions. The selectable gene may or may not have a 5′ promoter that is active in the packaging cell line, as well as in the transfected cell.

The recombinant retroviral vector DNA can be transfected into the amphotrophic packaging cell line Ψ-AM (see Cone and Mulligan, 1984) or other packaging cell lines which are capable of producing high titer stocks of helper-free recombinant retroviruses. After transfection, the packaging cell line is selected for resistance to G418, present at appropriate concentration in the growth medium. Adenoviral vectors (e.g. DNA virus vectors), particularly replication-defective adenovirus vectors, or adeno-associated vectors, have been described in the art (Kochanek *et al.*, 1996; Ascadi *et al.*, 1994; Ali *et al.*, 1994).

Nonviral gene transfer methods known in the art include chemical techniques, such as calcium phosphate co-precipitation, direct DNA uptake and receptor-mediated DNA transfer, and mechanical means, such as microinjection and membrane fusion-mediated liposomal transfer. In addition, viral-mediated gene transfer can be combined with direct *in vivo* gene transfer using liposomes, thereby allowing the delivery or the viral vectors to tumor cells, for example, and not to surrounding non-proliferating cells. A description of various liposomes which are stated as being useful for transferring DNA or RNA into cells is present in United States Patents 5,283,185 and 5,795,587. The retroviral vector producer cell line can also be injected directly into specific cell types, e.g., tumors, to provide a continuous source of viral particles, such as has been approved for use in patients afflicted with inoperable brain tumors.

Receptor-mediated gene transfer methods allow targeting of the DNA in the construct directly to particular tissues. This is accomplished by the conjugation of DNA (frequently in the form of a covalently closed supercoiled

quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. Using the amino acid sequences of the present invention for gene therapy may provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the invention. Both viral and non-viral methods are suitable. Examples of such transfer systems include, but are not limited to, delivery via liposomes or via viruses, such as adeno-associated or vaccinia virus. Numerous viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, adenovirus, vaccinia virus, adeno-associated virus, herpes viruses, including HSV and EBV, and retroviruses of avian, murine, and human origin). As is appreciated by those in the art, most human gene therapy protocols have been based on disabled murine retroviruses. Recombinant retroviral DNA can also be employed with amphotrophic packaging cell lines capable of producing high titer stocks of helper-free recombinant retroviruses (e.g., Cone and Mulligan, 1984).

A recombinant retroviral vector may contain the following parts: an intact 5′ LTR from an appropriate retrovirus, such as MMTV, followed by DNA containing the retroviral packaging signal sequence; the insulator element placed between an enhancer and the promoter of a transcription

useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999).

IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998; Apfel, 1999).

J. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration. Other modes of administration and examples of suitable formulative components for use with this embodiment are discussed below. Other modes of administration include intranasal, intrathecal, intracutaneous, percutaneous, enteral, and sublingual. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e. blood) of the recipient. Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or

I. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as potential therapeutics in pharmaceutical compositions, lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, and as research tools for further analysis of IR and IGF-1R. In particular, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, preferred amino acid sequence are: FHENFYDWFVRQVSK (D117, H2C), DYKDFYDAIQLVRSARAGGTRDKK (D118, 20E2), KDRAFYNGLRDLVGAVYGAWDKK (D119, 20C11), DYKDLCQSWGVRIGWLAGLCPKK (D116, JBA5), DYKDVTFTSAVFHENFYDWFVRQVSKK (D113, H2), and GRVDWLQRNANFYDWFVAELG (S175). More preferred IR agonists are: FHENFYDWFVRQVSK (D117, H2C) and GRVDWLQRNANFYDWFVAELG (S175). Most preferred is GRVDWLQRNANFYDWFVAELG (S175). Preferred dimer sequences are represented by S170, S171, S172, S232, S300 sequences (see Table 15).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast and prostate cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also

pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences which function as either agonists or antagonists at IR and/or IGF-1R. Examples of phage display libraries suitable for use in this invention include one such library containing randomized 40 amino acid peptides (RAPIDLIB™, Figure 16), another library containing rVab derived from human genomic antibody DNA (GRABLIB™, Figure 30). Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been described elsewhere (WO 96/04557; Mandecki *et al.*, 1997; Ravera *et al.*, 1998; Scott and Smith, 1990); Grihalde *et al.*, 1995; Chen *et al.*, 1996; Kay *et al.*, 1993, Carcamo *et al.*, 1998, all of which are incorporated herein by reference). Another phage display library suitable for use with this invention is available commercially from New England Biolabs (Ph.D. C7C Disulfide Constrained Peptide Library). Additional sequences may be obtained in accordance with the procedures described herein.

In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% BSA and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{3+} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides are generally unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g. by substituting each residue in turn). These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g. stereochemistry, bonding, size and/or charge), using data from a range of sources (e.g. spectroscopic techniques, X-ray diffraction data and NMR). Computational analysis, similarity mapping (which models the charge and/or volume of a

H. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557 which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides which bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region which has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display. See WO 96/04557 and Kay *et al.* (1996), both of which are incorporated herein by reference. The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

example, pRSET vectors (Invitrogen Corp., San Diego, CA). The expressed, tagged peptides can then be purified from a crude lysate of the cell-free translation system or host cell by chromatography on an appropriate solid-phase matrix.

Methods for directly purifying peptides from natural sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Naturally occurring peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

Antibody-based methods may also be used to purify naturally occurring or recombinantly produced peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

2. Chemical Synthesis Of Peptides

Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997). In addition, recombinant and synthetic methods of polypeptide production can be combined to produce semi-synthetic polypeptides.

carried out using established methods. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using techniques routinely practiced in the art.

Suitable cell-free systems for expressing recombinant peptides include, for example, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *Escherichia coli* (*E. coli*) S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). Such systems allow expression of recombinant polypeptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing coding regions and appropriate promoter elements.

Host cells for cloning vectors include bacterial, archebacterial, fungal, plant, insect and animal cells, especially mammalian cells. Of particular interest are *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, SF9, C129, 293, NIH 3T3, CHO, COS, and HeLa cells. These cells can be transformed, transfected, or transduced, as appropriate, by any suitable method including electroporation, $CaCl_2$-, LiCl-, LiAc/PEG-, spheroplasting-, Ca-Phosphate, DEAE-dextran, liposome-mediated DNA uptake, injection, microinjection, microprojectile bombardment, or other established methods.

For some purposes, it may be preferable to produce peptides in a recombinant system in which they carry additional sequence tags to facilitate purification. Non-limiting examples of tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS), GLU-GLU, and DYKDDDDK (FLAG®) epitope tags. Epitope tags can be added to peptides by a number of established methods. DNA sequences of epitope tags can be inserted into peptide coding sequences as oligonucleotides or through primers used in PCR amplification. As an alternative, peptide coding sequences can be cloned into specific vectors that create fusions with epitope tags; for

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region. Figure 17.

Notably, several fusion peptides show IR agonist activity as determined by an IR autophosphorylation assay (see Example 20). Figure 74. In particular, fusion peptides 439, 436, 449, and 463 show significant IR agonist activity (Figure 74).

G. Peptide Synthetic Techniques

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention.

1. Recombinant Synthesis

To obtain recombinant peptides, the corresponding DNA sequences may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see Sambrook *et al.*, 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

Cloning vectors for the expression of recombinant peptides include, but are not limited to, pUC, pBluescript (Stratagene, La Jolla, CA), pET (Novagen, Inc., Madison, WI), pMAL (New England Biolabs, Beverly, MA), or pREP (Invitrogen Corp., San Diego, CA) vectors. Vectors can contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host (e.g. antibiotic resistance), and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids, etc. Ligation of the coding sequences to transcriptional regulatory elements and/or to other amino acid coding sequences can be

TABLE 1

Fusion	Seq.	Action	Site	Fusion Concentration	MW (kDa)	K_d(HIR)
426	D8	N	2	0.76	52.2	1.4×10^{-6}
429	D8-6aa-D8	N-N	2-2	3.2	55.3	1.3×10^{-6}
430	H2C-6aa-RB6	A-	1-1	0.17	54.5	2.1×10^{-6}
431	H2C-6aa-F8	A-N	1-2	3.3	54.8	4.7×10^{-8}
432	H2C-12aa-F8	A-N	1-2	2.9	55.5	3.5×10^{-8}
433	H2C-9aa-F8	A-N	1-2	2.8	55.2	2.1×10^{-8}
434	G3-12aa-G3	N-N	1-1	0.01	56	3.2×10^{-6}
436	H2C-9aa-H2C	A	1-1	1.1	54.2	4.1×10^{-7}
437	H2C	N-N	1	0.3	51.5	8.3×10^{-6}
427	G3-6aa-G3	N-N	1-1	0.02	55.3	3.3×10^{-6}
435	H2C-3-H2C-3-H2C	A-A-A	1-1-1	2.1	55.5	2.0×10^{-6}
439	H2C-6aa-H2C	A-A	1-1	1.4	53.9	5.5×10^{-7}
449	H2C-12aa-H2C		1-1	1.5	51.8	6.2×10^{-7}
452	G3		1	0.15	48.8	7.8×10^{-7}
463	H2C-3aa-H2C	A-A	1-1	1.8	50.1	9.6×10^{-7}
464	LF-H2C		1	0.045	48.4	3.9×10^{-8}
446	LF-F8		2	1.9	49.1	7.7×10^{-7}
459	SF-RB6			0.069	48.1	7.7×10^{-8}
MBP*	lacZ			5.1	50	$> 1 \times 10^{-5}$

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ).
N = Antagonist
A = Agonist
LF = Long FLAG epitope (DYKDDDDK)
SF = Short FLAG epitope (DYKD)

Additional binding data for the fusion peptides are shown below:

Fusion	Highest conc. tested (μM)	Kd (HIR) μM
431-	0.2	0.033
431+	0.2	0.0074
432-	0.2	0.02
432+	0.2	0.0038
433-	0.2	0.03
433+	0.2	0.004

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs).

construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence.

In addition to producing the dimer peptides by recombinant protein expression, dimers may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

Other methods of constructing dimers include introducing a linker molecule which activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include diaminoproprionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula

$$O=CH-(CH_2)_n-CH=O,$$

Wherein n is 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Linkers may be used to link dimers either to the carboxyl terminal or the amino terminal.

2. Characterization Of Specific Dimers

Specific dimers which bind with high affinity to Site 1, Site 2, or both Site 1 and Site 2 of the insulin receptor are shown in Table 1. Although agonist activity has been observed for the Site 1-Site 1 dimers, the Site 1-Site 2 dimers may also possess desirable properties.

the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

In a preferred embodiment shown below (Figure 16)

MBP-FLAG-PEPTIDE-(G,S)n-PEPTIDE-E-TAG

a fusion construct producing a dipeptide comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a flag sequence to provide a cleavage site to remove the initial sequence. The peptide dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative

different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences which bind to the same site to form a multivalent ligand may be useful to produce molecules which are capable of cross-linking together multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 15).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition, combining amino acid sequences which bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

In one embodiment, hybrids of at least two peptides may be produced as recombinant fusion polypeptides which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of

REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

Clone	Sequence
F8	HLCVLEELFWGASLFGYCSG
F8-C12	**FQSLLEELVWGAPLFRYGTG**
F8-Des2	**PLCVLEELFWGASLFGYCSG**
F8-F12	PLCVLEELFWGASLFGQCSG
F8-B9	HLCVLEELFWGASLFGQCSG
F8-B12	DLRVLCELFGGAYVLGYCSE
NNKH-2B3	HRSVLKQLSWGASLFGQWAG
NNKH-2F9~	HLSVGEELSWWVALLGQWAR
NNKH-4H4~	APVSTEELRWGALLFGQWAG

D8-derived (Small C-C loop):

Clone	Sequence
D8	**KWLDQEWAWVQCEVYGRGCPSKK**
D8-G1	**QLEEEWAGVQCEVYGRECPS**
D8-B5~	ALEEEWAWVQVRSIRSGLPL
D8-A7	SLDQEWAWVQCEVYGRGCLS
D8-F1~	WLEHEWAQIQCELYGRGCTY

Midi C-C loop:

Clone	Sequence
D8-F10	GLEQGCPWVGLEVQCRGCPS
F8-B12~	DLRVLCELFGGAYVLGYCSE
F8-A9	PLWGLCELFGGASLFGYCSS

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides.

* Peptides synthesized and currently being purified

~ Peptides planned

F. Multivalent Ligands

This invention provides ligands which preferentially bind different sites on IR and IGF-1R. The amino acid motifs which bind IR at one site (Site 1, Figure 13) are A6, B6, revB6, and F2. A second in site (Site 2, Figure 13) binds F8 and D8. Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences which bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF):

Clone	Sequence
G3	KRGGGTFYEWFESALRKHGAGKK
H2	VTFTSAVFHENFYDWFVRQVSKK
H2C	FHENFYDWFVRQVSKK
A6S-IR3-E12	GRVDWLQRNANFYDWFVAELG
A6S-IR4-G1	NGVERAGTGDNFYDWFVAQLH
H2CB-R3-B12	QSDSGTVHDRFYGWFRDTWAS
20E2A-R3-B11	GRFYGWFQDAIDQLMPWGFDP
rB6-F6	RYGRWGLAQQFYDWFDR
E4Dα-1-B8-IR-	GFREGQRWYWFVAQVT
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV
H2CB-R3-D2	WTDVDGFHSGFYRWFQNQWER
H2CB-R3-D12	VASGHVLHGQFYRWFVDQFAL
H2CB-R4-H5	QARVGNVHQQFYEWFREVMQG
H2C-B-E8*	TGHRLGLDEQFYWWFRDALSG
H2CB-3-B6-IR-	VGDFCVSHDCFYGWFLRESMQ
A6S-IR2-C1	RMYFSTGAPQNFYDWFVQEWD

B6-like (FYxxLxxL):

Clone	Sequence
20C11	KDRAFYNGLRDLVGAVYGAWDKK
20E2	DYKDFYDAIDQLVRGSARAGGTRDKK
B62-R3-C7	EHWNTVDPFYFTLFEWLRESG
B62-R3-C10	EHWNTVDPFYQYFSELLRESG
20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWWDQG
20E2-B-E3*	IQGWEPFYGWFDDVVAQMFEE
20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC
RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS
RPNN-4-F3-HOLO*	HYNAFYEYFQVLLAETW
20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER
RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT
20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG
20E2-B-E12	GNSGGSFYRYFQLLLDSDGMS
20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC

Reverse B6-like (LxxLxxYF):

Clone	Sequenc
rB6-A12	LDALDRLMRYFEERPSL
rB6-F9	PLAELWAYFEHSEQGRSSAH
rB6-4-E7-IR	LDPLDALLQYFWSVPGH
rB6-4-F9-IR	RGRLGSLSTQFYNWFAE
rB6-E6	ADELEWLLDYFMHQPRP
rB6-4-F12-IR	DGVLEELFSYFSATVGP

Group 6 (WPxYxWL):

Clone	Sequence
R20β-4-A4-IR	WPGYLFFEEALQDWRGSTED

Peptides by design**:

Clone	Sequence
H2C-PD1-IR-	AAVHEQFYDWFADQYKK
A6S-PD1-IR-	QAPSNFYDWFVREWDKK
20E2-PD1-IR-	QSFYDYIEELLGGEWKK
B6C-PD1-IR-	DPFYQGLWEWLRESGKK

Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence which is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R.

A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X_{95}. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X_{11}. Tryptophan at X_{13} also favors selectivity of IR. A tryptophan amino acid at X_{13} rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X_{15} favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X_{23} is essentially required for IR binding. In addition, tryptophan at X_{31} is also highly preferred. At X_{32}, glycine is preferred for IR selectivity.

E. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 13, competition data (See Example 15) indicates that peptides comprising the A6, B6, revB6, and F2 motifs compete for binding to the same site on IR (Site 1) whereas the F8 and D8 motifs compete for a second site (Site 2). Similarly, the decrease of dissociation of B6 motif peptide (20E2) from IGF-1R by a D8 ligand indicates multiple interacting binding sites.

The identification of peptides which bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 15.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

MOTIF 10 (Group6):

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAWPAQARGRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	3.6	34.9	0.1	9.7

Clon	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
B6Hα-3-E8-IR	RGKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8	0.2
B6Hα-3-F10-IR	GCVVEWQKWHGASDPFYHKLSELGGCS	47.2	8.8	1.9	4.6	0.2
B6Hα-2-D6-IR	GRTMAVMAAGGPDDPFYHKLSELVQGG	33.5	4.4	1.0	4.4	0.2
B6Hα-3-E7-IR	GCAVVEEAERSRGDPFYHKLSELIQGC	47.0	5.6	1.3	4.3	0.2
B6Hα-2-D1-IR	GCEVIVEEGDSADPFYHKLSELCQGS	11.7	5.4	1.3	4.2	0.2
20E2A-3-D10-IGFR	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	LSVALSFYDALGQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
B6Hα-4-G8-IR	GGTKAVAKVGTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6	0.3
B6L-4-D7-IR	AETSVQVGWIRLQSVWPGEHWNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6Hα-1-A3-IR	SRAKVEAEMPDSGDPFYHKLSELLASG	37.4	2.6	0.8	3.3	0.3
B6Hα-3-F7-IR	SRVAATKEKRPSDDPFYHKLSELLQGS	41.5	3.1	1.0	3.1	0.3
B6Hα-2-D8-IR	SSETAKMVTGTRDDPFYHKLSELVQGS	19.3	3.0	1.0	3.0	0.3
B6Hα-1-B3-IR	GCITAENGAGDPFYHKLSELGGCS	33.1	3.2	1.1	2.9	0.3
B6Hα-3-E5-IR	RCGDEEGWQENRRDDPFYHKLSELFGGC	28.8	2.9	1.0	2.9	0.3
20E2A-4-G11-IGFR	MNVFVSFYDAIDQLVCQRIGC	20.7	3.3	1.3	2.6	0.4
20E2Bβ-3-C7-IR	QSGSGDFYDWLSRLIRGNGDG	1.5	3.1	1.5	2.0	0.5
B6Hα-3-E6-IR	CGAKMTGTPNDPFYHKLSELLQRG	18.2	2.3	1.2	1.9	0.5
20E2A-3-A3-IGFR	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
B6L-4-A7-IR	AGTPAQVG*NRLWSVWPGEHWNTVDPFYNKLSELLRESGA	11.6	3.4	1.9	1.8	0.6
B6Hα-3-F1-IR	CSMAAVAEAGDDDDPFYHKLSELCQGS	22.5	2.4	1.3	1.8	0.5
B6L-3-G6-IR	VDTPAQVGWNRLWSVGPGEHWYTDDPFYH*LSELLRESGA	7.6	2.5	1.8	1.4	0.7
B6L-3-G5-IR	AETSAQVGWQRLWSVWPGDHWSTLDPFYHKLSELLRESGA	11.5	2.0	1.4	1.4	0.7
20E2A-3-A4-IGFR	AGSVTSFYDAMEQLVATGTSA	16.8	2.5	1.8	1.4	0.7
B6-PD1-IGFR	TDDGFYDALEQLVQGSKK					
20E2-PD1-IGFR(RP10)	GSFYEALQRLVGGEQGKK					

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20E2B-1-A11-IGFR	RFSTDGFYQYLLALVGGGPVG	15.0	9.5	1.0	9.7	0.1
20E2B-3-D4-IGFR	NSRDGGFYLQLERLLGFPVTG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	VVTPVNFYRALEALVRG.RLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	QPAPDGFYSALMKLIGRGGVS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	PGTDLGFYQALRCVVIQGACD	11.7	4.9	0.6	8.1	0.1
20E2B-4-F10-IGFR	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	QPDHSYFYSLLQELVGSEERL	11.9	14.7	1.9	7.7	0.1
20C-3-A4-IGFR	QFYGCLLDLSLGVPSFGWRRRCITA	17.7	8.8	1.2	7.6	0.1
20E2B-3-D11-IGFR	LGVTDGFYAALGYLIHGVGQF	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	CMM.DGFYAGLGCLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	ICTGQGFYQVLCGLLRGTSAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	QGNVLDFYGWIGRLLAKQGSD	10.3	6.2	0.9	7.3	0.1
20E2B-3-E12-IGFR	VATSQGFYSGLSELLQGGGNV	13.9	6.0	0.8	7.3	0.1
20E2B-2-B8-IGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	0.8	7.2	0.1
NNRPγ-4-A9-IR	EGSGFYGYFFSLLGLQG	3.0	10.0	1.4	7.1	0.1
20E2B-4-G11-IGFR	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	0.6	7.0	0.1
20E2B-3-D6-IGFR	DSVGDNFYQLLESLVGGHGVG	20.7	17.8	2.6	6.9	0.1
B6Hα-2-C7-IR	RGIVAMVEATEVGSDHDPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1
20E2B-2-B7-IGFR	LSSDGQFYRALNLLLQGSAGR	18.0	6.1	0.9	6.7	0.1
20E2B-3-C4-IGFR	ASSASGFYELLQRLAGLGLEV	23.4	20.4	3.3	6.2	0.2
20C-3-E4-IGFR	FFYRCLSRLLGGQLGSRLGLSCIGD	37.7	7.7	1.3	6.0	0.2
NNRPγ-4-A1-IR	IIGGFYSYFNSVLRLGT	9.7	10.9	1.8	6.0	0.2
20E2B-4-H8-IGFR	PAGPCGFYCGLGLLLHGDQSP	7.2	5.3	0.9	5.9	0.2
20E2B-4-H9-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	0.9	5.6	0.2
B6Hα-2-C10-IR	SGAKVIVVTGDSGDPFYHKLSELLQGS	46.9	5.8	1.1	5.3	0.2
20E2A-3-C7-IGFR	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1	0.2
20E2B-1-A8-IGFR	TLRSPTFYDWLEMVLTHGQGG	16.1	4.4	0.9	5.0	0.2
NNRPγ-4-A7-IR	RFDPFYSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2

MOTIF 2 (B6-like):

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	TFYSCLASLLTGTPQPNRGPWERCR	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	FFYDCLAALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
B6Hα-1-B5-IR	CCTTEMVVMDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
R20β-4-A6-IR	RGQSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLGLGPSG	18.6	18.1	1.1	16.8	0.1
R20α-4-20A12-IR	RLFYCGIQALGANLGYSGCV	48.6	39.9	2.4	16.6	0.1
20E2Bβ-4-G7-IR	LQPCSGFYECIERLIGVKLSG	19.9	25.2	1.6	15.8	0.1
NNRPγ-4-B11-IR	LKDGFYDYFWQRLHLGS	4.1	18.7	1.2	15.5	0.1
20E2B-3-C6-IGFR	VEGRGLFYDLLRQLLARRQNG	17.9	16.8	1.1	14.8	0.1
B6Hα-1-A2-IR	RGCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
20E2A-4-F11-IGFR	QGGSASFYDAIDRLLRMRIGG	21.3	18.8	1.3	14.6	0.1
B6Hα-3-E9-IR	RCEEKQAEVGPSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
20C-3-F6-IGFR	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20E2B-4-H3-IGFR	KLHNLMFYYGLQRLVWGAGLG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	0.6	13.8	0.1
20C-3-A1-IGFR	SSYGCDGFYLMLFSLGLVASQELEC	26.5	20.8	1.5	13.7	0.1
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
R20α-3-20E2-IR	FYDAIDQLVRGSARAGGTRD	46.3	39.9	3.1	12.9	0.1
20E2B-4-H12-IGFR	YSCGDGFYSLLSDLLGGQFRC	6.5	9.7	0.8	12.8	0.1
B6Hα-3-F11-IR	RGMKEEVLVGGSTDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	LRIANLFYQRLWDLAFGGGG	15.7	16.7	1.5	11.1	0.1
B6Hα-2-C4-IR	RCGRW*AEMGAGDDPFYHKLSELVCG	20.7	9.9	0.9	11.0	0.1
R20α-4-20C11-IR	DRAFYNGLRDLVGAVYGAWD	43.7	30.8	3.0	10.3	0.1
20E2B-4-F8-IGFR	PVGVQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1

Clone	Sequence	Ratios over Background E-Tag	IGF-1R	IR	Comparisons IGF-1R/IR	IR/IGF-1R
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5	0.3
E4Dα-2-D11-IR	GFREGSFYDWFQAQVT	40.2	11.1	3.3	3.4	0.3
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTVHSA	22.4	6.2	1.9	3.3	0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2	0.5
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5
A6-PD1-IGFR	SFHEDFYDWFDRQVSGSLKK					
H2C-PD1-IGFR(RP9)	GSLDESFYDWFERQLGKK					

IGF-1R-SELECTIVE SEQUENCES

MOTIF 1 (A6-like):

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1
H2CA-4-F5-IGFR	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFLRQVAESSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFERQVSHQRGVR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDWFVRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYEWFVRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEWFVRQVSLSLKGK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
E4Dα-4-H2-IR	GFREGNFYEWFQAQVT	37.8	33.9	8.2	4.1	0.2
H2CA-4-F7-IGFR	SSIGGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	QSPVGSSHEDFYDWFFRQVAQSGAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDWFDRQVSQSGGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDWFVCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3

helix, it is important to maintain the spacing of those residues along the amino acid sequence. For example, the second and third amino acids of B6 (X_7 and X_8) are oriented at opposite sides of the helix. See Figure 12.

D. IR Binding Preferences

As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in Figures 1A-10I. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as X_{98} X_{99} B6 X_{100}. X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X_{98} and a proline at X_{99} is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X_{100}, an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

3. Formula 3

An amino terminal extension of Formula 3 defined as X_{101} X_{102} X_{103} revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

C. Secondary Structure

Without being bound by theory, it is believed that the B6 and reverse B6 motifs participate in alpha helix formation such that the most highly preferred residues at positions X_6, X_7, X_{10} and X_{13} (B6) and X_{14} X_{17} X_{20} and X_{21} (rB6) reside on the same side of a helix. See Figure 12. Because both B6 and RB6 motifs form structurally analogous motifs from their palindrome sequences, the use of D-amino acids instead of typical L-amino acids would be expected to produce amino acid sequences having similar properties to the L-amino acid sequences. D-amino acids may be advantageous, as the resultant sequences may be more resistant to enzymatic degradation than L-amino acid sequences. In addition, to maintain the appropriate orientation of highly preferred amino acid sequences on the appropriate side of the

preferred IR or IGF-1R consensus sequences which do not correspond to one of the motifs described above. Such sequences are described in Figures 10C-10I.

B. Amino And Carboxyl Terminal Extensions Modulate Activity of Motifs

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions which allow for binding to IR, IGF-1R or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 X_{93} X_{94} X_{95} X_{96} X_{97} wherein X_{93} may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X_{94} and X_{97} are any amino acid; X_{95} is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X_{95} however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X_{95} may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X_{96} is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X_{96} may be used to enhance IR selectivity.

X_{114}) ends immediately flanking X_{107}-X_{111} are preferably a cysteine residue, most preferably at X_{105} and X_{113} respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X_{104} and X_{114} are both small amino acids such as, for example, alanine and glycine. Most preferably, X_{104} is alanine and X_{114} is glycine. X_{105} may be any amino acid but is preferably valine. X_{112} is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG. The IR binding displayed amino acid sequences are described in Figure 8.

9. An amino acid sequence comprising DYKDLCQSWGVRIGWLAGLCPKK (Formula 9, JBA5). The Formula 9 motif is another motif believed to form a cysteine loop which possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic. See Figure 11A. Binding of Formula 9 through IGF-1R is detected by ELISA. Nonlimiting examples of Formula 9 amino acid sequences are described in Figures 9A-9C.

10. WX_{123} GYX_{124} WX_{125} X_{126} (Formula 10, Group 6 Secondary Library) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. Nonlimiting examples of Formula 10 amino acid sequences are described in Figures 10A-10B.

11. Other Motifs

Another motif for use with this invention includes WPGY. Examples of specific peptide sequences comprising this motif include KVRGFQGGTVWPGYEWLRNAAKK (E8), and KSMFVAGSDRWPGYGVLADWLKK (F2).

Various amino acid sequences which bind IR and/or IGF-1R have been identified through panning of various libraries designed to identify

most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is X_{62} L X_{64} X_{65} X_{66} W X_{68} X_{69} X_{70} X_{71} C X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} C X_{80} X_{81}.

Nonlimiting examples of Formula 6 amino acid sequences are described in Figures 6A-6E.

7. HX_{82}, X_{83}, X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92} (Formula 7) wherein X_{82} is proline or alanine but most preferably proline; X_{83} is a small amino acid more preferably proline, serine or threonine and most preferably proline; X_{84} is selected from leucine, serine or threonine but most preferably leucine; X_{85} is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X_{86} may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X_{87} is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid X_{88}, X_{89} and X_{90} may be any amino acids; X_{91} is an aliphatic amino acid with a strong preference for leucine as is X_{92}. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is $HPPLSX_{86}LX_{88}X_{89}X_{90}LL$. The Formula 7 motif binds to IR with little or no binding to IGF-1R.

Nonlimiting examples of Formula 7 amino acid sequences are described in Figure 7.

8. Another sequence is X_{104}, X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114}. (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X_{106} through X_{111} are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end. Accordingly, the most preferred sequence for X_{107} X_{108} X_{109} X_{110} X_{111} is WPTYW. At least one of the three amino acids on the amino terminal (X_{104}, X_{105} X_{106}) and at least one of the amino acids carboxy terminal (X_{112} X_{113}

acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 4A-4E list nonlimiting examples of Formula 4 amino acid sequences.

5. X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} $X_{56}X_{57}$ $X_{58}X_{59}X_{60}$ X_{61} ("mini F8", Formula 5) wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60} and X_{61} are any amino acid. X_{43}, X_{46}, X_{49}, X_{50} and X_{54} are hydrophobic amino acids, however, X_{43} and X_{46} are preferably leucine, whereas X_{50} is preferably phenylalanine or tyrosine but most preferably phenylalanine. X_{47} and X_{59} are cysteines. X_{48} is preferably a polar amino acid, i.e. aspartic acid or glutamic acid, but most preferably glutamic acid. Use of the small amino acid at position 54 may confer IGF-1R specificity. X_{51}, X_{52} and X_{57} are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is X_{42} X_{43} X_{44} X_{45} LCEX_{49} FGGX_{53} X_{54} X_{55} X_{56} GX_{58} CX_{60} X_{61}. Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR. Nonlimiting examples of Formula 5 amino acid sequences are described in Figure 5.

6. X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} (Formula 6, "D8") wherein X_{62}, X_{65}, X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} may be any amino acid. X_{66} may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of X_{66} with glutamine or valine may result in attenuation of binding. X_{63}, X_{70}, and X_{74} are hydrophobic amino acids. X_{63} is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. X_{70} and X_{74} are preferably valine, isoleucine, leucine, or methionine. X_{74} is most preferably valine. X_{64} is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. X_{67} and X_{75} are aromatic amino acids. Whereas tryptophan is highly preferred at X_{67}, X_{75} is preferably tyrosine or tryptophan but most preferably tyrosine. X_{72} and X_{79} are cysteines which again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence. D8 is

acid is strongly preferred at X_{18}. See Figures 3A-3D for nonlimiting examples of Formula 3 amino acid sequences.

4. $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ (Formula 4, "F8") wherein X_{22}, X_{25}, X_{26}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{36}, X_{37}, X_{38}, X_{40}, and X_{41} are any amino acid. X_{35} and X_{37} may be any amino acid when the F8 motif is used as an IR binding ligand or as a component of an IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at X_{37} and a hydrophobic amino acid, particularly, leucine, is preferred at X_{35}. X_{23} is a hydrophobic amino acid. Methionine, valine, leucine or isoleucine are preferred amino acids for X_{23}, however, leucine which is most preferred for preparation of an IGF-1R binding ligand is especially preferred for preparation of an IR binding ligand. At least one cysteine is located at X_{24} through X_{27}, and one at X_{39} or X_{40}. Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions X_{24} and X_{39} if the cysteines occupy other positions. In one embodiment, for example, the cysteine at position X_{24} may occur at position X_{27} which will produce a smaller loop provided that the cysteine is maintained at position X_{39}. These smaller looped peptides are described herein as Formula 5, infra. X_{27} is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position X_{27} decreases binding to IR but has less of an effect on binding to IGF-1R. X_{31} is any aromatic amino acid and X_{32} is any small amino acid. For binding to IGF-1R, glycine or serine are preferred at position X_{31}, however, tryptophan is highly preferred for binding to IR. At position X_{32}, glycine is preferred for both IGF-1R and IR binding. X_{36} is an aromatic amino acid. A preferred consensus sequence for F8 is X_{22} LC X_{25} X_{26} E X_{28} X_{29} X_{30} WG X_{33} X_{34} X_{35} X_{36} X_{37} X_{38} C X_{40} X_{41} whereas the amino

preferably glycine, aspartic acid, glutamic acid, asparagine or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine.

X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine.

X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid.

X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid.

X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid.

X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid.

X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine.

X_{122} is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid.

Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

Nonlimiting examples of amino acid sequences having the Formula 2 and 2A motifs are described in Figures 2A-2P.

3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14}, X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15}, X_{16}, X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably phenylalanin . For use as an IGF-1R binding ligand, an aromatic amino

at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 11A. Two amino acid sequences comprising the A6 motif possess agonist activity at IR are FHENFYDWFVRQVSKK (D117; H2C) and GRVDWLQRNANFYDWFVAELG-NH_2 (S175). Nonlimiting examples of Formula 1 amino acid sequences are shown in Figures 1A-1O.

2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, the B6 motif) wherein X_6 and X_7 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_6 is phenylalanine and X_7 is tyrosine. X_8, X_9, X_{11} and X_{12} may be any amino acid. X_{10} and X_{13} are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X_{10} is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X_{13} is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are $FYX_8\ X_9\ L\ X_{11}\ X_{12}L$, $FYX_8\ X_9\ IX_{11}\ X_{12}\ L\ FYX_8\ AIX_{11}\ X_{12}L$, and $FYX_8\ YFX_{11}\ X_{12}\ L$.

Another Formula 2 motif for use with this invention comprises $FYX_8\ YFX_{11}\ X_{12}\ L$ and is shown as Formula 2A ("NNRP") below:

$X_{115}\ X_{116}\ X_{117}\ X_{118}\ FY\ X_8\ YF\ X_{11}\ X_{12}\ L\ X_{119}\ X_{120}\ X_{121}\ X_{122}$,

wherein X_{115}-X_{118} and X_{118}-X_{122} may be any amino acid which allows for binding to IR or IGF-1R. X_{115} is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are

The amino acids within each of these defined groups may be substituted for each other in the motifs described below, subject to the specific preferences stated herein. In addition, synthetic or non-naturally occurring amino acids may also be used in accordance with this invention.

Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which amino acid residues are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids such as lysine which promote the stability of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

Peptides that bind to IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley et al., U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.

A. Consensus Motifs

The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1, the A6 motif) wherein X_1, X_2, X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF and FYEWF. The A6 motif possesses agonist activity

acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

A further consideration in identifying peptides provided by this invention for use as therapeutics is the relative activity at either IR or IGF-IR. Thus, a peptide which has efficacy at IR and clinically insignificant activity of IGF-IR may be a useful therapeutic even though such a peptide may bind IGF-IR with relatively high affinity.

At least ten different binding motifs have been identified which bind to active sites on IR; at least four of these also bind to IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T).

reported to be important for binding and activity, this invention surprisingly provides amino acid sequences which define common binding motifs on IR and IGF-1R which are capable of conferring agonist and/or antagonist activity at these receptors. In addition, this invention identifies multiple binding sites (Sites 1 and 2) on IR and IGF-1R which appear to be allosterically coupled.

Although capable of binding to IR and/or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are neither based on insulin or IGF-1 native sequences, nor do they reflect an obvious homology to any such sequence.

The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to one of the receptors is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e. non-naturally occurring peptides or polypeptides. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR or IGF-1R sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide or protein provided by this invention should have an affinity (K_d) of between about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about 10^{-12} M. Most preferably, the affinity is 10^{-9} to about 10^{-11} M. For use as a reagent in a competitive binding assay to identify other ligands, the amino

Figure 69: Competition of MBP-peptide fusion proteins to rVab 12H10 binding to insulin receptor. Four MBP-peptides fusion proteins at indicated concentrations were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figures 70A-70N: Peptide binding displacement curves showing the displacement of ^{125}insulin or ^{125}IGF-1 from HIR or HIGF-1R in the presence of various peptides.

Figures 71A-71Z; 71A2-71Z2; 71A3-71B3: Concentration dependent modulation of ^{3}H-glucose into adipocytes by various peptides. Formula 1 motif peptide responses are shown in Figures 71A-71V; 71A2-71J2; Formula 9 motif peptide response is shown in Figures 71W-71Z; Formula 2 motif peptide response is shown in Figures 71K2-71L2; Miscellaneous peptide motif 10 peptide responses are shown in Figures 71M2-71P2; Formula 6 motif peptide response is shown in Figure 71Q2-Figure 71R2; and Formula 4 motif peptide response is shown in Figure 71S2-Figure 71W2. Formula 1 and Formula 2 motif peptide response is shown in Figure 71X2-Figure 71A3. Fusion peptide S291 response is shown in Figure 71B3.

Figures 72A and 72B: Competition of Site 1(Figure 72B) and Site 2 (Figure 72A) phage displayed peptides with recombinant cleaved dipeptides.

Figure 73: Competition of IGF-1R, peptide H2C (D117), peptide C1 (D112), and peptide RP6 (20C-3-G3-IGFR) in a homogeneous fluorescent-resonance energy transfer assay based on the binding of IGF-1R to peptide 20E2 (D118).

Figure 74: Stimulation of IR autophosphorylation *in vivo* by MBP-fusion peptides.

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs which bind to the IGF-1 receptor (IGF-1R) and/or the insulin receptor (IR). In addition to binding to IR and IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at one or both of these receptors. Based on the differing regions of IR and IGF-1R which are

containing cysteine residues. The hatched bars indicate value is ≤ 54 % control value. Figure 54B. Competition with fusion proteins containing the consensus sequence. The notation, c-c, indicates phage displayed peptides with cysteine residues. Figure 54C. Competition with fusion protein containing a control peptide.

Figure 57: Nucleotide and predicted amino acid sequence of the gene encoding the 6f6 rVab that binds to IR.

Figure 58: Nucleotide and predicted amino acid sequence of the gene encoding the 14c8 rVab that binds to IR.

Figure 59: Comparison of the VH CDR3 sequences of different rVabs that bind to IR, and competitions of these rVabs and insulin for binding to IR.

Figure 60: Biological response of insulin, rVab 12h10, and rVab 13h9 in 32D cells expressing or not expressing IR.

Figure 61: Competition of rVab 6f6 and insulin for binding to IR.

Figure 62: Competition of rVab 6f6 and IGF-1 for binding to IR.

Figure 63: Competition of synthetic peptides and soluble rVab antibodies for binding of biotinylated peptides to insulin receptor. Synthetic peptides or soluble rVab at indicated concentrations were incubated with biotinylated peptides overnight using the heterogeneous TRFA.

Figure 64: Binding of C1 to IR and IGF-1R.

Figure 65: Competition of peptides for binding to IR.

Figure 66: H2C competition for b-peptide binding to IR. Biotinylated peptides at indicated concentrations were competed by increasing concentrations of H2C for binding to IR using the heterogeneous TRFA.

Figure 67: C1C competition for b-C1 binding to IR. Biotinylated C1 peptide at 0.3 μM was competed by increasing concentrations of C1C for binding to IR using the heterogeneous TRFA.

Figure 68: Competition of peptides for binding of rVab 12H10 to insulin receptor. Synthetic peptides at indicated concentration were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figure 48: Biopanning results and sequence alignments of Groups 2 through 7 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor.

Figure 49A-49D: Dose response curve of D118 peptide (Formula 2 motif) stimulated increase of ^{3}H-glucose into mouse adipocytes.

Figures 50A-50D: Titration of the synthetic peptides C1 (Figures 50A, 50C) or B6 (Figures 50B, 50D) against constant concentration of phage bound to IR (Figures 50A, 50B) or IGF-1R (Figures 50C, 50D). Phage are represented by: open circle – 20D3; open square – 20A4; open triangle – 20E2; open diamond – F2; filled circle – F8; and filled square – D8.

Figure 51A-51D: Titration of the IGF-1R synthetic peptides against constant concentration of phage. Symbols for the peptides are: open circles – H2; filled circles – H2C; open square – C1; filled square – C1C; open triangle – D2C; filled triangle – E4; open diamond – A6; and filled diamond p53.

Figure 52A-52D: Hill plot analysis of phage clones. The detailed data are provided in Table 7. Symbols are the same as in Figure 51.

Figure 53: Competition between the insulin and the IR-binding phage. The results for seven different groups (categories) of phage binders are shown.

Figure 54: Titration of the synthetic peptide 20A4 against constant concentration of phage. Phage binding to IR are represented by: open circle – 20D3; filled circle B8; open square – 20A4; filled square – D8; open up triangle – 20E2; open down triangle – D10; filled down triangle – A2; open diamond – F2; filled diamond – E8; and cross-filled circle – F8.

Figure 55: A schematic drawing for the construction of protein fusions of the maltose binding protein and peptides from phage libraries.

Figure 56A-56C: Insulin Receptor Competition ELISA using MBP-Peptide Fusion Proteins. Figure 54A. Competition with fusion proteins

Figure 37: Nucleotide sequence of gene encoding the M100 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 38: Nucleotide sequence of gene encoding the 46A7 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 39: Nucleotide sequence of gene encoding the 49E8 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 40: Assay results demonstrating the binding of soluble forms of three rVabs to IGF-1R.

Figure 41: Assay results showing that the 43G7 rVab stimulates growth of IGF-1R$^+$ cells.

Figure 42: Assay results showing that the stimulation by rVab 43G7 is antagonized by the 1G2P, 49E8, and 46A7 rVabs. The assay was done on IGF-1R$^+$ cells.

Figure 43: Eu-based fluorescence assay results showing that the binding of peptide 5.1 to IGF-1R can be competed by the IGF-1 ligand.

Figure 44: Results of the time-resolved fluorescence assay showing that the binding of 43G7 rVab to IGF-1R is effectively competed by IGF-1.

Figure 45: Eu-based fluorescence assay showing that the binding of the B6 peptide to IGF-1R is effectively competed by the 43G7 rVab.

Figures 46A-46D: Results of the Eu-based fluorescence assay showing that the binding of the europium-labeled 43G7 rVab to IGF-1R is effectively competed by selected scAbs specific for IGF-1R.

Figure 47: Biopanning results and sequence alignments of Group 1 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: ++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

Figures 26A and 26B: Design of the secondary phage library A6S based on the Motif 1 peptide sequences. Figure 26A: Sequence design for the A6S secondary phage library. Figure 26B: Synthetic oligonucleotide for the A6L secondary library. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 27: Sequences of the five H5-like peptides that show agonistic activity toward IGF-1R. The C-terminal lysine contains a biotin moiety linked to the amino group of the side chain.

Figure 28: Listing of amino acid sequences obtained from panning with the A6S library.

Figure 29: Listing of amino acid sequences obtained from panning with the H5 secondary phage library.

Figure 30: Schematic of the genomic rVab library.

Figure 31: Listing of the V_H, kappa and lambda genes used to assemble the rVab antibody library for IGF-1R binders.

Figure 32. Schematic of the assembly of the single-chain IGF-I and insulin antibody libraries from restriction fragments.

Figure 33: Sequences of the restriction fragments used to assemble the rVab libraries.

Figure 34: Nucleotide sequence of the gene encoding the 43G7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 35: Nucleotide sequence of the gene encoding the 1G2P rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 36: Nucleotide sequence of gene encoding the 39F7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 19: DNA sequences of the frameshifted clones.

Figures 20A and 20B: Results of the phage ELISA for binding to IGF-1R. Wells were coated with 100 ng/well IGF-1R and blocked. Competitor, the IGF-1 native ligand, was present prior (1 h) and during the phage incubation (1 h). Phage were detected with HRP-anti M13 phage antibody and reported as OD_{405} as described. Total Binding is shown in Figure 20A and Percent Inhibition is shown in Figure 20B.

Figure 21: Sequences of the designed IGF-1R-specific synthetic peptides.

Figure 22: Assay results showing that Motif 2 peptides (5.1 and 5.2) antagonize the effects of IGF-1 on IGF-$1R^+$ cells.

Figure 23: Assay results showing that Motif 1 peptides (5.3 and 5.4) stimulate growth of IGF-$1R^+$ cells. Cells expressing human IGF-1R (30,000 cells per well) were incubated with the 5.4 peptide for 42 h at 37°C. Experiments were done in triplicate. Background signal A_{450}=0.15. Proliferation was measured using WST-1 reagent (Boehringer Mannheim Biochemicals/Roche Molecular Biochemicals, Indianapolis, IN).

Figures 24A and 24B: Demonstration of binding of peptide 5.1 to IGF-1R using BIAcore. Figure 24A: Binding as a function of the peptide concentration. Figure 24B: Inhibition of IGF-1 binding by peptide 5.1. RU – refractive units.

Figures 25A and 25B: Design of the secondary phage library A6L based on the Class II peptide sequences. Figure 25A: Design of the sequence of the gene. Underlined residues indicate positions mutated to optimize the codons for expression in *E. coli*. Figure 25B: Synthetic oligonucleotide for the A6L secondary library. Underlined residues were doped in the chemical DNA synthesis. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 10G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide as indicated (F815) panned against IGF-1R.

Figure 10H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IR.

Figure 10I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IGF-1R.

Figure 11A: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 11B: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 12: Illustration of helix wheels applied to Formula 2 and 3 motifs.

Figure 13: Illustration of 2 binding site domains on IR based on competition data.

Figure 14: Dissociation of 20E2 peptide from IGF-1R in the presence of buffer (filled circle), 30 μM IGF-1 (open circle), 100 μM H2C (filled square), 100 μM 20E2 (filled triangle), 100 μM D8 (B12; open square), 100 μM C1 (filled, inverted triangle) and 100 μM RPG (filled diamond).

Figure 15: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

Figure 16: Schematic diagram of the phage-displayed peptide library. The peptide is displayed as a protein fusion to the N-terminus of gene *III* encoding the minor coat protein of the phage.

Figure 17: BIAcore analysis of competition binding between IR and MBP fusion H2C-9-H2C, H2C and H2C-3-H2C.

Figure 18: Sequence alignments of Class I and Class II peptides. The Class I peptides have been shown to be IGF-1R antagonists, while the Class II peptides are IGF-1R agonists.

Figure 6E: Formula 6 motif peptide sequences identified from other libraries panned against IR.

Figure 7: Formula 7 motif peptide sequences.

Figure 8: Formula 8 motif peptide sequences identified from a commercial phage display peptide library and synthetic sequences. Small letters denote D-amino acids. Unnatural amino acids are denoted with a 3-letter abbreviation in certain sequences. K_d values greater than 2×10^{-5} are approximate.

Figure 9A: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the H5 peptide as indicated (referred to as "H5") panned against IGF-1R.

Figure 9B: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IGF-1R.

Figure 9C: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IR.

Figure 10A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R.

Figure 10B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR.

Figure 10C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR.

Figure 10D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR.

Figure 10E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 10F: Miscellaneous peptide sequences identified from a $X_{1-4}CX_{6-20}$ and panned against IGF-1R.

Figure 3D: Formula 3 motif peptide sequences identified from a library constructed using the sequence $X_3LXXLXXYFX_{12-17}$ (reverse B6; rB6) panned against IGF-1R.

Figure 4A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 4B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IR.

Figure 4C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 4D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (20% dope; referred to as "F820") panned against IR.

Figure 4E: Formula 4 motif peptide sequences identified from other libraries panned against IR.

Figure 5: Formula 5 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 6A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR.

Figure 6B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (15% dope; referred to as "D815") panned against IR.

Figure 6C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IR.

Figure 6D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IGF-1R.

Figure 2I: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IGF-1R.

Figure 2J: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IR.

Figure 2K: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IGF-1R.

Figure 2L: Formula 2 motif peptide sequences identified from a library constructed using the sequence X_{1-6}FYDAIDQLVX_{16-21} (20E2-A) panned against IR.

Figure 2M: Formula 2 motif peptide sequences identified from a library constructed using the sequence X_{1-6}FYDAIDQLVX_{16-21} (20E2-A) panned against IGF-1R.

Figure 2N: Formula 2 motif peptide sequences identified from a library constructed using the sequence X_{1-6}FYXXhXXhhX_{16-21} (20E2-B) panned against IR.

Figure 2O: Formula 2 motif peptide sequences identified from a library constructed using the sequence X_{1-6}FYXXhXXhhX_{16-21} (20E2-B) panned against IGF-1R.

Figure 2P: Formula 2 motif peptide sequences identified from a library constructed using the sequence X_{1-6}FYRYFXXLLX_{16-21} (NNRP) panned against IR.

Figure 3A: Formula 3 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 3B: Formula 3 motif peptide sequences identified from a $X_{1-4}CX_{6-20}$ library panned against IGF-1R.

Figure 3C: Formula 3 motif peptide sequences identified from a library constructed using the sequence X_3LXXLXXYFX_{12-17} (reverse B6; rB6) panned against IR.

Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHENFYDWFVRQVSX_{21-26} (H2C-A) panned against IGF-1R.

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHXXFYXWFX_{16-21} (H2C-B) and panned against IR.

Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHXXFYXWFX_{16-21} (H2C-B) and panned against IGF-1R.

Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR.

Figure 2A: Formula 2 motif peptide sequence identified from a random 40mer library panned against IR.

Figure 2B: Formula 2 motif peptide sequences identified from a random 40mer library panned against IGF-1R.

Figure 2C: Formula 2 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 2D: Formula 2 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 2E: Formula 2 motif peptide sequences identified from a $X_{1-4}CX_{6-20}$ library panned against IGF-1R.

Figure 2F: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IR.

Figure 2G: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IGF-1R.

Figure 2H: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IR.

Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR.

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R.

Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR.

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R.

Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10}NFYDWFVX$_{18-21}$ (also referred to as "A6S") panned against IR.

Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10}NFYDWFVX$_{18-21}$ (also referred to as "A6S") panned against IGF-1R.

Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IR.

Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IGF-1R.

Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IR.

Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IGF-1R.

Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHENFYDWFVRQVSX$_{21-26}$ (H2C-A) panned against IR.

therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

Another object of this invention is to provide structural information derived from the amino acid sequences of this invention which may be used to construct other molecules possessing the desired activity at the relevant IR of IGF-1R binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-10G. Amino acid sequences comprising the motif of Formulas 1 through 10. Sequences were identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R.

The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

Symbols in the listed sequences are: Q - TAG Stop; # -TAA Stop; * - TGA Stop; and ? - Unknown Amino Acid. It is believed that a W replaces the TGA Stop Codon when expressed. Except for the 20C, A6L, and B6L libraries, all libraries are designed with the short FLAG Epitope DYKD (Hopp *et al.*, 1988) at the N-terminus of the listed sequence and AAAGAP at the C-terminus. The 20C, A6L, and B6L libraries have the full length FLAG epitope DYKDDDDK.

different sites on one receptor or to cause receptor complexing through different sites.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin. Such compounds may act as antagonists or agonists of insulin function in cell based assays.

This invention also provides amino acid sequences such as peptides and recombinant antibody variable regions (rVab) that inhibit binding of insulin to the insulin receptor. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds that mimic insulin.

This invention also provides kits for identifying compounds that bind to the insulin receptor. The invention further provides therapeutic compounds that bind the insulin receptor.

In another embodiment, this invention provides assays for identifying compounds which mimic the binding characteristics of IGF-1. Such compounds act as antagonists or agonists of IGF-1 hormone function in cell based assays.

The invention also provides amino acid sequences such as peptides and rVabs which inhibit binding of IGF-1 to IGF-1R. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds which mimic IGF-1.

Another embodiment of this invention is the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the genes encoding the amino acid sequences which bind at IR or IGF-1R and possess agonist or antagonist activity.

It is an object of this invention to provide amino acid sequences which bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR and/or IGF-1R.

It is a further object of this invention to provide specific amino acid sequences which possess agonist, partial agonist or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as

i. an amino acid sequence comprising the sequence DYKDLCQSWGVRIGWLAGLCPKK.

j. $WX_{123}GYX_{124}WX_{125}X_{126}$ wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid.

In one embodiment, preferred amino acid sequences FYX_3WF ("A6" motif) and $FYX_8X_9L/IX_{11}X_{12}L$ ("B6" motif) have been identified which competitively bind to sites on IR and IGF-1R and possess either agonist or antagonist activity. Surprisingly FYX_3WF which possesses agonist activity at IGF-1R, can possess agonist or antagonist activity at IR. Similarly, $FYX_8X_9L/IX_{11}X_{12}L$, which is an antagonist at IGF-1R, possesses agonist activity at IR.

This invention also identifies at least two distinct binding sites on IR and IGF-1R based on the differing ability of certain of the peptides to compete with one another and insulin or IGF-1 for binding to IR and IGF-1R. Accordingly, this invention provides amino acid sequences which bind specifically to one or both sites of IR and/or IGF-1R. Furthermore, specific amino acid sequences are provided which have either agonist or antagonist characteristics based on their ability to bind to the specific sites of IR.

In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R may be covalently linked together to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences may be covalently bound together to form homo- or heterocomplexes. Dimers of the same amino acid sequence, for example, may be used to form receptor complexes bound through the same corresponding sites. Alternatively, heterodimers may be used to bind to

c. $X_{14}\,X_{15}\,X_{16}\,X_{17}\,X_{18}\,X_{19}\,X_{20}\,X_{21}$ wherein X_{14}, and X_{17} are hydrophobic amino acids, X_{15}, X_{16}, X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

d. $X_{22}\,X_{23}\,X_{24}\,X_{25}\,X_{26}\,X_{27}\,X_{28}\,X_{29}\,X_{30}\,X_{31}\,X_{32}\,X_{33}\,X_{34}\,X_{35}\,X_{36}$ $X_{37}\,X_{38}\,X_{39}\,X_{40}\,X_{41}$ wherein X_{22}, X_{25}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{36}, X_{37}, X_{38}, X_{40}, and X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X_{25} and X_{40} X_{31} and X_{32} are small amino acids.

e. $X_{42}\,X_{43}\,X_{44}\,X_{45}\,X_{46}\,X_{47}\,X_{48}\,X_{49}\,X_{50}\,X_{51}\,X_{52}\,X_{53}\,X_{54}\,X_{55}\,X_{56}$ $X_{57}\,X_{58}\,X_{59}\,X_{60}\,X_{61}$ wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60} and X_{61} may be any amino acid, X_{43}, X_{46}, X_{49}, X_{50}, X_{54} are hydrophobic amino acids, X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51}, X_{52} and X_{57} are small amino acids.

f. $X_{62}\,X_{63}\,X_{64}\,X_{65}\,X_{66}\,X_{67}\,X_{68}\,X_{69}\,X_{70}\,X_{71}\,X_{72}\,X_{73}\,X_{74}\,X_{75}\,X_{76}$ $X_{77}\,X_{78}\,X_{79}\,X_{80}\,X_{81}$ wherein X_{62}, X_{65}, X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80}, and X_{81} may be any amino acid; X_{63}, X_{70}, X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop.

g. H $X_{82}\,X_{83}\,X_{84}\,X_{85}\,X_{86}\,X_{87}\,X_{88}\,X_{89}\,X_{90}\,X_{91}\,X_{92}$ wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine, serine or threonine, X_{85} is a polar amino acid, X_{86}, X_{88}, X_{89} and X_{90} are any amino acid, and X_{87}, X_{91} and X_{92} are an aliphatic amino acid.

h. $X_{104}\,X_{105}\,X_{106}\,X_{107}\,X_{108}\,X_{109}\,X_{110}\,X_{111}\,X_{112}\,X_{113}\,X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111}, and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104}, X_{105} and X_{106} and at least one of X_{112}, X_{113} and X_{114} are cysteine; and

III. SUMMARY OF THE INVENTION

This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR and/or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules which bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

In one aspect of this invention large numbers of peptides have been screened for their IR or IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. At least ten generic amino acid sequences have been identified which bind IR and IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1, X_2, X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid;

b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids;

desirable to identify other molecular structures which mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, none of these efforts has resulted in finding a successful drug.

Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996) reports the isolation of a monomer peptide antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage panning procedures.

Wrighton *et al.* (1996) and Livnah *et al.* (1996) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimers which enabled the dimerization two EPO receptors.

Most recently, Cwirla *et al.* (1997) reported the identification of two families of peptides that bind to the human thrombopoietin (TPO) receptor and are competed by the binding of the natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand .

WO 96/04557 reports the use of peptides and antibodies which bind to active sites of biological targets and which are then used in competition assays to identify small molecules which are agonist or antagonists at the biological targets.

Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin. (Williams *et al.*, 1995). When these residues are introduced into the equivalent site of the IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in the IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding. (Mynarcik *et al.*, 1996).

Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to the IGF-1 receptor but that those contacts differ from those that insulin utilizes to bind to the insulin receptor. (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems." (De Meyts, 1994).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth.

It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be

making the mature IGF-1 slightly larger than insulin. (Blakesley, 1996). The C region of human insulin-like growth factor (IGF-1) appears to be required for high affinity binding to the type I IGF receptor. (Pietrzkowski *et al.*, 1992). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R receptor. (Pietrzkowski *et al.*, 1992). A further structural distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize. (De Meyts, 1994).

The α-subunits, which contain the ligand binding region of the IR and IGF-1R, demonstrate between 47-67% overall amino acid homology. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid homology.

Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α-subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ. (Blakesley, *et al.*, 1996). For example, the cysteine-rich domain of the IGF-1R (amino acids 191-290) was determined to be essential for high-affinity IGF binding, but not insulin binding by introducing this IGF-1R region into the corresponding region of the IR (amino acids 198-300) and observing that the IR demonstrated high affinity binding of IGF-1 while maintaining high-affinity insulin binding. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the affinity for IGF-1 was not detectable while the affinity for insulin remained undisturbed.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions.

be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998). The L domains consist of a single-stranded right-handed β-helix (a helical arrangement of β-strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

The β-subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α-subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994).

To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

IGF-1 and insulin competitively cross-react with IGF-1R and IR. (Schäffer, 1994). Despite 45% overall amino acid homology, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor. (Mynarcik, *et al.*, 1997). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands. (Blakesley *et al.*, 1996).

Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, whereas the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain,

composed of two α and two β subunits which form a disulfide-linked heterotetramer (β-α-α-β). They have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. Besides IR and IGF-1R, the other known member of the IR family is the insulin-related receptor (IRR), for which no natural ligand is known.

While similar in structure, IGF-1 and insulin receptors serve different physiological functions. The IR is primarily involved in metabolic functions whereas the IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to the IGF-1 receptor, and IGF-1 its metabolic effects through the insulin receptor, remains controversial. (De Meyts, 1994).

The insulin receptor is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved yielding the disulfide-linked monomer α-β insulin receptor. Two α-β monomers are linked by disulfide bonds between the α-subunits to form a dimeric form of the receptor (β-α-α-β-type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine rich region (amino acids 156-312) (Ward *et al.*, 1995). Many determinants of insulin binding seem to reside in the α-subunit. A unique feature of the insulin receptor is that it is dimeric in the absence of ligand.

The sequence of IR is highly homologous to the sequence of the type-1 insulin-like growth factor receptor (IGF-1R). The homology level varies from about 40% to 70%, depending on the position within the α-subunit. The three-dimensional structures of both receptors may therefore

repair, and recently has been implicated in various forms cancer including prostrate, breast, colorectal, and ovarian. It is similar in size, sequence and structure to insulin, but has 100-1,000-fold lower affinity for the insulin receptor (Mynarcik *et al.*, 1997).

Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997; Crowne *et al.*, 1998), amyotropic lateral sclerosis (Lai *et al.*, 1997), and diabetic motor neuropathy (Apfel and Kessler, 1996). Other potential therapeutic applications of IGF-1 such as osteoporosis (Canalis, 1997), immune modulation (Clark, 1997) and nephrotic syndrome (Feld and Hirshberg, 1996) are being examined.

A number of studies have analyzed the role of natural IGF-1 in various disease states. Most interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995; Figueroa *et al.*, 1995; Torring *et al.*, 1997). Additionally, elevated serum IGF-1 levels have been connected with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan *et al.*, 1998). Recent studies have indicated a connection between IGF-1 and other cancers such as breast, colorectal, and ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in Conover, 1996 and Rajaram *et al.*, 1997). Interestingly, PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (Cohen *et al.*, 1992; Cohen *et al.*, 1994; Lilja, 1995). Clearly, regulation of IGF-1R activity can play an important role in several disease states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

The type-1 insulin-like growth-factor receptor (IGF-1R) and insulin receptor (IR) are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular structures are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of the level of glucose at a whole body level. This effect by insulin occurs predominantly in liver, fat, and muscle. In liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat, insulin stimulates glucose uptake, storage, and metabolism. Disruptions of glucose utilization are very common in the population in giving rise to diabetes.

Signal transduction in target cells is initiated by binding of insulin to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of the receptor, which are transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of the insulin receptor's tyrosine kinase, and the binding of soluble effector molecules that contain SH2 domains such as phophoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase Cγ to IR (Lee and Pilch, 1994).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and

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(54) Title: INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites which are present on insulin and insulin-like growth factor receptors which selectively bind the peptides of this invention. As agonists, certain of the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonists may also be developed as therapeutics.